NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*: THE KEY TO PREVENTING STAPHYLOCOCCAL DISEASE

Nasal carriage of *Staphylococcus aureus*: the key to preventing staphylococcal disease
Jan Kluytmans.
Thesis Erasmus University Rotterdam, The Netherlands
ISBN 90-9009776-7
NUGI 742
Subject headings: *Staphylococcus aureus; surgical site infection; epidemiology; mupirocin.*
Cover design by Dick Bezem
Lithografie by DataLitho bv, Aalsmeer, The Netherlands
Typografie by MegaSet bv, Aalsmeer, The Netherlands
Printing by MurKostverloren bv, Aalsmeer, The Netherlands

NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*: THE KEY TO PREVENTING STAPHYLOCOCCAL DISEASE

NEUSDRAGERSCHAP VAN *STAPHYLOCOCCUS AUREUS*:
DE SLEUTEL TOT DE PREVENTIE VAN STAPHYLOCOCCEN INFECTIES

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. dr. P.W.C. Akkermans M.A.
en volgens besluit van het college voor promoties.

De openbare verdediging zal plaatsvinden op
woensdag 30 oktober 1996 om 13.45 uur

door

Johannes Adrianus Jacobus Wilhelmus Kluytmans
geboren te Oisterwijk

PROMOTIECOMMISSIE

Promotor:	Prof. Dr. H. A. Verbrugh
Promotor:	Prof. Dr. M. F. Michel
Overige leden:	Prof. Dr. H. A. Bruining Prof. Dr. J. Dankert Prof. Dr. C. M. J. E. VandenBroucke-Grauls
Copromotor:	Dr. J. H. T. Wagenvoort

Aan mijn ouders

CONTENTS

Introduction 1

Chapter 1: Surgical wound infections in cardio-thoracic surgery

Part 1: Surveillance of postoperative infections in thoracic surgery 7
Part 2: Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery 17
Part 3: Reduction of surgical site infections in cardio-thoracic surgery by elimination of nasal carriage of *Staphylococcus aureus* 25
Part 4: Cost-effectiveness of perioperative mupirocin nasal ointment in cardio-thoracic surgery 35

Chapter 2: Bacteremia in patients on hemodialysis

Part 1: Prevention of *Staphylococcus aureus* bacteremia in hemodialysis patients by elimination of nasal carriage using mupirocin 47

Chapter 3: Epidemiology

Part 1: Global epidemiology of methicillin-resistant *Staphylococcus aureus* 55
Part 2: Epidemiology and risks associated with nasal carriage of *Staphylococcus aureus* 61

Chapter 4: Molecular biological typing methods

Part 1: Multicenter evaluation of arbitrarily primed PCR for typing of *Staphylococcus aureus* strains 91
Part 2: Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and genotyping 109

Summary and conclusions 127

Publications 135

Curriculum vitae 138

INTRODUCTION

environment. Strains which cause outbreaks in hospitals are often resistent against commonly used antimicrobial agents, e.g. β-lactams. In chaper 3 the extensive literature on this subject was critically reviewed. The focus in part 1 was on the current epidemiology of methicillin-resistant *S. aureus* and in part 2 on nasal carriage. The epidemiology, underlying mechanisms, identification of patients at risk of infection and effects of elimination strategies are discussed.
To elucidate the epidemiological behaviour of *S. aureus* it is essential to have reliable typing methods available. Recently developed molecular typing techniques seem highly promising. Several of these genotyping techniques are evaluated in chapter 4. In a multicenter study the reproducibility of the arbitrary primer polymerase chain reaction was evaluated using a welldefined collection of *S. aureus* strains (part 1). In part 2 this technique, and several others, were used to evaluate and succesfully control an outbreak of multi-resistant *S. aureus* in the University Hospital Rotterdam.

REFERENCES

1 **Boyce JM, White RL, Spruill EY.** Impact of methicillin-resistant *Staphylococcus aureus* on the incidence of nosocomial staphylococcal infections. *J Infect Dis* 1983;**148**:763

2 **Calia FM, Wolinsky E, Mortimer EA, Abram JS, Rammelkamp CH.** Importance of the carrier state as a source of *Staphylococcus aureus* in wound sepsis. *J Hyg Camb* 1969;**67**:49-57.

3 **Dolman CE.** Clinical uses of staphylococcal toxoid. *Lancet* 1935;**1**:306-13.

4 **Emori TG, Gaynes RP.** An overview of nosocomial infections. *Clin Microbiol Rev* 1993;**6**:428-42.

5 **Finland M, Jones JWF.** Staphylococcal infections currently encountered in a large municipal hospital: some problems in evaluating antimicrobial therapy in such infections. *Ann N Y Acad Sci* 1957;**65**:191-205.

6 **Fleming A.** A comparison of the activities of antiseptics on bacteria and leucocytes. *Proc Roy Soc* 1924;**96**:171-80.

7 **Fleming A.** On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae. Br J Exp Path* 1929;**10**:226-30.

8 **Jevons MP.** "Celbenin" resistant staphylococci. *Br Med J* 1961;**i**:124-5.

9 **Kluytmans JAJW, Mouton JW, IJzerman EPF, Vandenbroucke-Grauls CMJE, Maat AWPM, Wagenvoort JHT, Verbrugh HA.** Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery. *J Infect Dis* 1995;**171**:216-9.

10 **Law MR, Gill ON, Turner A.** Methicillin-resistant *Staphylococcus aureus*: associated morbidity and effectiveness of control measures. *Epidemiol Infect* 1988;**101**:301-9.

11 **Lister J.** On a new method of treating compound fracture. *Lancet* 1867;**1**:326-8.

12 **Mudd S.** Staphylococcal infections in the hospital and community. *JAMA* 1958;**166**:1177-8.

13 **Ogston A.** Report upon micro-organisms in surgical diseases. *Br Med J* 1881;**1**:369-75.

14 **Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, Martone WJ.** The national nosocomial infection surveillance system. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975-1991. *Infect Cont Hosp Epidemiol* 1992;**13**:582-6.

15 **Rosenbach JF.** Mikroorganismmen bei den Wund-Infektions-Krankheiten des Menschen. *Wiesbaden* 1884.

16 **Skinner D, Keefer CS.** Significance of bacteremia caused by *S. aureus*; study of 122 cases and review of the literature concerned with experimental infection in animals. *Arch Intern Med* 1941;**68**:851-75.

17 **Spink WW.** Infectious Diseases. Prevention and treatment in the Nineteenth and Twentieth Centuries. *Minneapolis: university of Minnesota Press*, 1978.

18 **Stamm AM, Long MN, Belcher B.** Higher overal nosocomial infection rate because of increased attack rate of methicillin-resistant *Staphylococcus aureus. Am J Infect Control* 1993;**21**:70-4.

19 **Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I.** Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect Dis* 1994;**13**:50-5.

20 **Voss A, Doebbeling BN.** The worldwide prevalence of methicillin-resistant *Staphylococcus aureus. Int J Antimicrobial Agents* 1995;**5**:101-6.

21 **Weinstein HJ.** The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. *New Engl the J Med* 1959;**260**:1303-8.

22 **White A.** Increased infection rates in heavy nasal carriers of coagulase-positive staphylococci. *Antimicrob Agents Chemother* 1963;30:667-670.

23 **Williams REO, Patricia-Jevons M, Shooter RA, Hunter CJW, Girling JA, Griffiths JD, Taylor GW.** Nasal staphylococci and sepsis in hospital patients. *Br Med J* 1959;**Oct**:658-62.

24 **Williams REO, Blowers R, Garrod LP, Shooter RA.** Hospital infection, causes and prevention. 1966, 2nd edition. *Lloyd-Luke (medical books) Ltd.*

25 **IJzerman EPF, Boelens HAM, Tjhie JHT, Kluytmans JAJW, Mouton JW and Verbrugh HA.** ΔAPACHE II for predicting course and outcome of nosocomial *Staphylococcus aureus* bacteremia and its relation to host defense. *J Infect Dis* 1996;**173**:914-9.

CHAPTER ONE
SURGICAL WOUND INFECTIONS IN CARDIO-THORACIC SURGERY

PART ONE
SURVEILLANCE OF POSTOPERATIVE INFECTIONS IN THORACIC SURGERY

JAJW Kluytmans, JW Mouton, APWM Maat, MAAJ Manders, MF Michel and JHT Wagenvoort.

Adapted from: *Journal of Hospital Infection* 1994;**27**:139-47

SUMMARY

Postoperative infections (PIs) are serious complications of thoracic surgery. To gain insight into the nature and the scope of the problem, an 18 month prospective surveillance was conducted at the department of thoracic surgery of the University Hospital Rotterdam, Dijkzigt. PIs were classified according to CDC criteria. One hundred and ninety-four out of 983 patients (19.7%) developed one or more PIs and in these 194 patients, 268 PIs were diagnosed. The incidence of PIs was 2.0 per 100 days of postoperative stay. The mean postoperative length of stay (LOS) of the 194 patients with PIs was 14.1 days longer than those without PIs. Deep surgical wound infections (DSWIs) were associated with the longest prolongation of the median postoperative LOS in the hospital (30 days longer). Although lower than DSWIs, incisional surgical wound infections also had a significant prolongation of stay (median 10 days longer). *Staphylococcus aureus* was the most important pathogen associated with surgical wound infections (SWIs). Phage typing of 29 strains causing SWI showed only two identical pairs, so only a minority of infections could be explained by cross-infection. Older age, and more complicated procedures (e.g. cardiac valve operations) were independent, statistically significant risk factors for the development of PIs. Since there is a progressive trend towards operating on older patients and performing more complicated procedures, the incidence of PIs is expected to increase. Therefore it will become increasingly important to develop new strategies to prevent these serious complications.

INTRODUCTION

Open heart surgery is a prime example of the advances in medicine over the last decade and these procedures are now performed routinely on a large scale. In the US approximately 652,000 open heart surgical procedures were carried out in 1988.[1] In spite of modern surgical techniques, perioperative antibiotic prophylaxis and meticulous care, postoperative infections (PIs) remain frequent complications. The consequences of these complications can be severe. Apart of the suffering of patients and prolongation of their hospital stay,[6,11] infections are associated with significant mortality.[10]
In order to target preventive measures, it is necessary to gain insight into the nature and the scope of the problem.[7] We therefore conducted an 18-month prospective surveillance study in adult patients, who were operated on at the department of thoracic surgery of the University Hospital Rotterdam, Dijkzigt in The Netherlands.

PATIENTS AND METHODS

From August 1989 until February 1991 a prospective surveillance for PIs was performed at the department of thoracic surgery of the University Hospital Rotterdam, Dijkzigt. This is a tertiary referral center for cardiac and pulmonary surgery. The department consists of an operating theater, a postoperative intensive care unit and a postoperative ward.
The day before, surgery patients washed their body and hair with povidone-iodine or with chlorhexidine soap. Hair removal was performed using depilatory creams. Perioperatively, patients were given cefuroxime for 24 h as systemic antibiotic prophylaxis. The loading dose of 1.5 g was given 1 h before the incision. Subsequent doses of 750 mg were given every 6 h. If patients reported an allergy to β-lactam antibiotics, clindamycin (600 mg every 8 h) was given instead. After surgery all patients were taken to the intensive care unit for at least 24 h.
During the surveillance period the ward was visited by an investigator (JK or MM). The medical records of patients were studied for the presence of PIs using criteria as defined for this purpose by the Centers for Disease Control (CDC).[5] These criteria are based upon both clinical and laboratory parameters. Hospital identification number, sex, age, admission, operation, and discharge date, type of operation, surgeon and first assistant were recorded.
In Table 1 the types of operation are shown: coronary artery bypass grafting (CABG) operations were divided into procedures in which the saphenous vein (SV) was used as graft and in procedures in which one or both of the internal mammary arteries (IMAs), whether or not in combination with the SV, were used. Cardiac valve operations (CVOs) were considered alone or in combination with CABG procedures. Lung operations, mainly performed for exploration and/or resection of malignancies, constituted a separate group. The last group were operations that did not belong to one of the categories mentioned above (OTH). This group consisted mainly of operations for congenital heart disorders and dissecting aortic aneurysms.
If PIs were present, the site, date of onset, and the microorganism(s) cultured were recorded. According to the CDC criteria, the sites of PI were divided into urinary tract infections (UTIs), respiratory tract infections (RTIs), primary bloodstream infections (PBIs), incisional surgical wound infections (ISWIs), deep surgical wound infections (DSWIs) and other infections. The following intervals were calculated:

- Length of stay: number of days from admission date until discharge or death;
- Postoperative length of stay: number of days from operation date until discharge or death;
- Pre-infection length of stay: number of days from operation date until onset of postoperative infection;

For the calculation of the incidence of surgical wound infections (SWIs), the following corrections were made: Firstly, if a patient had both an ISWI and a DSWI at different sites, this patient was counted only as a DSWI, and secondly if one patient had two or more ISWIs or DSWIs at different sites, this patient was counted only once.
If *Staphylococcus aureus* was isolated, phage typing was performed at the National Institute of Public Health and Environmental Hygiene (RIVM, Bilthoven, The Netherlands).
The results were analyzed using the SAS statistical package.[12] Differences between groups were determined by Student's *t*-test. Logistic regression analysis with forward and backward elimination was used to determine dependence between risk factors. The following variables were put into the model: sex, age, preoperative length of stay, and type of procedure (Table 1). Statistical significance was accepted at $P< 0.05$ (two-tailed).

RESULTS

From August 1989 until February 1991 we evaluated 983 patients. The majority, 696 (70.8%), were males. The mean age was 59.9 years (range 16-87) for males and 60.7 years (range 15-83) for females. The mean length of stay was 14.9 days (range 5-125). The total postoperative length of stay of 13,395 days. Table 1 shows the number and percentage of the various kinds of operations which were performed during the study period. More than half of the procedures (61.1%) involved CABG. In 60% of the CABG-procedures the IMA was used as graft. Nearly a quarter (23.9%) of the procedures involved cardiac valve replacement.

Table 1: *Number (N) and percentage of the different kinds of operations performed on patients during the study period.*

Kind of operation	N	%
CABG• using saphenous veins only	198	20.1
CABG• using internal mammary artery	342	34.8
*CVO**	174	17.7
*CVO** and CABG• using saphenous vein only	38	3.9
*CVO** and CABG• using internal mammary artery	23	2.3
Lung operation	113	11.5
OTH†	95	9.7
Total	983	100.0

• CABG = Coronary Artery Bypass Graft
* CVO = Cardiac Valve Operation
† OTH = Other procedures

In 194 patients (19.7%), a total of 268 PIs were diagnosed (average: 1.4 PI per infected patient, range 1-5). The incidence of PI was 2.0 per 100 days of postoperative stay.
Table 2 shows the mean with standard deviation and the median of the postoperative length of stay in patients without and with PIs. The mean postoperative length of stay was 14.1 days longer in patients with PI. The median was 7 days longer. In Table 3 the frequencies of the various PIs are shown with the accompanying mean and median postoperative and pre-infection length of stay. All sites were associated with a prolongation of postoperative length of stay. The longest prolongation of stay was associated with the DSWIs and the group of other PIs.

Table 2: *Post-operative length of stay in days: Mean with Standard Deviation (SD) and median in patients without and with postoperative infections.*

		Postoperative length of stay in days		
Patients	N+	Mean	SD	Median
Without infection	789	10.8	5.5	11
With infection	194	24.9	21.1	18

+ N = number of patients

Table 3: *Postoperative and pre-infection length of stay in days: mean with Standard Deviation (SD) and median in patients with different sites of postoperative infections.*

		Length of stay in days					
		Postoperative			Pre-infection		
Site of infection	N+	Mean	SD	Median	Mean	SD	Median
Urinary tract	82	29.1	27.9	19	12.0	15.6	6
Respiratory tract	89	23.7	21.6	17	5.9	8.1	3
Incisional surgical wound	37	23.5	14.2	21	11.9	10.3	9
Deep surgical wound	38	50.7	31.4	41	12.3	10.0	10
Primary bloodstream	14	27.9	14.3	24	13.1	11.6	9
Other sites	8	56.9	44.1	45	16.9	17.4	10

+ N = number of patients

Table 4 shows the frequency of isolation of various microorganisms from the different sites of infections. From 47 of the 268 (17.5%) sites of infection there was either no culture performed or no pathogen isolated. From the remaining 221 sites of infection, 236 pathogens were isolated. *S. aureus* was isolated from 12 of the 37 ISWIs (32.4%), and from 20 of the 38 DSWIs (52.6%). Five of the DSWIs with *S. aureus* were associated with a secondary bacteraemia. *S. aureus* was isolated from 32 of the 75 SWIs (42.7%). All *S. aureus* isolates were susceptible to methicillin and aminoglycosides. Phage typing was performed on 29 of these 32 isolates. In this group of 29 isolates only two identical pairs were found. The phage types of the other 25 strains were unique.

In Table 5 the crude relative risks (RR) and the 95% confidence intervals (CI) of various risk factors for the development of PIs and for the development of SWIs are shown. Women, CVO and OTH were associated with a significantly increased RR for the development of PIs (Table 5). Also the mean age of patients with PIs (63.2 years) was significantly higher than for patients without PIs (59.3 years, $P = 0.0001$). None of the surgeons or first-assistants were associated with significantly increased or decreased RR (results not shown). To identify independent variables for the development of PIs, logistic regression analysis was performed. Using this method the following variables showed statistical significance: older age ($P = 0.0001$), CVO ($P = 0.005$) and OTH ($P = 0.0001$). The risk factors for the development of SWI were analyzed separately. The development of SWI was only significantly associated with OTH (Table 5). The mean age for patients with SWI was 61.8 years and for patients without SWI, 59.9 years ($P > 0.05$). None of the surgeons or first-assistants were associated with significantly increased or decreased RR (results not shown). Logistic regression analysis showed that OTH was the only independent variable ($P = 0.0006$).

Table 4: *Frequency of isolation of micro-organisms from various sites of postoperative infection.*

	Site of infection+						
Microorganism	UTI	RTI	ISWI	DSWI	PBI	Other	All
Staphylococcus aureus	0	4	12	20	1	1	38
Coagulase negative *Staphylococcus*	0	0	5	9	1	1	16
Enterococcus spp.	10	1	1	2	0	2	16
Streptococcus pneumoniae	0	8	0	0	0	0	8
Haemophilus influenzae	0	20	0	0	0	0	20
Aerobic Gram-negative rods	55	18	12	6	9	4	104
Pseudomonas aeruginosa	7	3	1	1	2	2	16
Other pathogen	3	8	2	1	3	1	18
No pathogen isolated or no culture performed	10	32	2	2	0	1	47
All	85	94	35	41	16	12	283

+ Abbreviations used for the different sites of infection are explained in the patients and methods section.

DISCUSSION

One of the objectives of this study was to determine the incidence of PIs after thoracic surgery. This study showed an incidence of 268 PIs in 983 patients (27.3%). The incidence was 2.0 postoperative infections per 100 days postoperative stay. Rates found in other studies show large variations. There are many reasons for these variations. Firstly, variations of infection rates in different institutes are caused by numerous factors, including the patient population,[2] the kind of antibiotic prophylaxis,[4,9] the method of preoperative hair-removal,[13] and the use of the IMA as graft.[14,15] There are also differences caused by bias, such as the reliability of the surveillance method or the criteria used for the diagnoses of PI. Results from different studies are therefore difficult to compare with each other. This matter is discussed in more detail by Doebbeling *et al.*[4]

The mean postoperative length of stay of the 194 patients with one or more PIs was 14.1 days longer than of the patients without infection (Table 2). PIs were thus associated with a total of 2,735 days of extra length of stay. This was 20.4% of the total postoperative length of stay. Nelson *et al.*[11] found an extra length of stay associated with PI in patients undergoing open-heart surgery of 16.7 days, which compares well with our findings. Table 3 shows that all sites of PI were associated with a prolongation of stay. Because of the large standard variations of the mean, it is more appropriate to consider the median for comparison of the different sites. OTH and DSWIs were associated with the longest median postoperative length of stay (45 and 41 days respectively). Since OTH was a relatively small and heterogeneous group, DSWI is the most interesting group to look at in more detail. The 38 patients with DSWI alone accounted for 1,516 extra days of postoperative length of stay. This was 55.4% of the total extra length of stay associated with PIs. Although the prolongation of stay was most pronounced in DSWI, ISWI also had a median length of stay which was 10 days longer than in uninfected patients. It appears that even these "minor" infections were associated with considerable extra length of stay. The median pre-infection length of stay was short compared with the median postoperative length of stay for all sites. Thus, although infections began shortly after operation, the length of stay after the onset was relatively long. This strongly suggests that PIs contributed to the prolongation of stay. This causal relation is also shown in the following comparison: The median pre-infection length of stay of ISWI was almost identical to DSWI (9 vs. 10 days). However the median postoperative length of stay for the more severe infections (DSWIs) was 20 days longer, most likely as a result of these infections. Prolongation of postoperative stay due to SWI after cardiovascular surgery was

also found in other studies. Doebbeling[4] found a prolongation of 10.4 days for ISWIs and of 29.6 days for DSWIs. The median length of hospital stay for patients with SWIs in a study by Loop *et al.*[10] was 41 days. Our findings confirm that SWIs after thoracic surgery are serious complications, associated with a significant prolongation of the postoperative stay.

Table 5: *Crude relative risks (RR) and 95% confidence intervals (CI) of various risk factors for the development of postoperative infections and surgical wound infections.*

	Postoperative infections		Surgical wound infections	
Risk factor	RR	(CI)	RR	(CI)
Gender:				
women (as opposed to men)	1.31	(1.01 - 1.70)	1.03	(0.64 - 1.66)
Procedure:				
Cardiac valve operation (compared with CABG)	1.48	(1.10 - 1.70)	0.59	(0.30 - 1.16)
Lung operation	0.94	(0.62 - 1.41)	1.07	(0.55 - 2.08)
other[+]	1.71	(1.23 - 2.37)	2.58	(1.55 - 4.30)
Procedures involving IMA[*] (compared to SV[†] only)	0.99	(0.70 - 1.42)	1.09	(0.60 - 1.98)

[+] other are the procedures described in the patients and methods section as other.
[*] IMA is the Internal Mammary Artery.
† SV is the Saphenous Vein.

Table 4 shows the frequency of isolation of different microorganisms from various sites of PI. More than 95% of the patients received a urinary catheter perioperatively, which explains the incidence of UTI. Most UTIs were caused by aerobic Gram-negative rods and *Enterococcus spp.* as was to be expected. Also the majority of PBIs were caused by aerobic Gram-negative rods. From RTIs a broader spectrum of microorganisms was isolated. *Streptococcus pneumoniae* and *Haemophilus. influenzae* were not isolated from sites other than the respiratory tract. The diagnosis of RTI was largely based on clinical findings. This is indicated by the high percentage (36.0%) in which there was either no culture performed or no pathogen isolated. The majority of the patients with postoperative RTI had clinical signs of infection and the production of purulent sputum, which fulfilled the criteria used in this study.[5] However most of these infections resolved either spontaneously or with more intensive postoperative physical therapy, but without antibiotic treatment. This indicates that most of these RTIs were not classical pneumonia's but rather postoperative collections of pulmonary secretions which could usually be resolved by expectoration. Nevertheless these infections were associated with a significant prolongation of postoperative length of stay, as can be seen in Table 3.

The types of microorganisms isolated from ISWIs was somewhat different from those isolated from DSWIs. The most important differences were that aerobic Gram-negative rods were more often isolated from ISWI (32.4% vs. 15.8%), whereas *S. aureus* was more often isolated from DSWI (52.6% vs. 32.4%). Most of the ISWIs were infections of the venectomy site on the leg. The location near the groins and perineal region might explain the greater portion of aerobic Gram-negative rods in this group. The greater amount of *S. aureus* in DSWI, sometimes with secondary bacteraemia, is typical of this.[16] To identify cross-infections or common sources of SWI with *S. aureus*, phage typing was performed. For this analysis 29 isolates were available. Only two pairs of identical isolates were identified. The majority (93.1%) of cases could therefore not be explained by cross-infection or the presence of a common source. In the 1950s and 60s several epidemiological studies indicated that nasal carriers of *S. aureus* have an increased risk for the development of SWI.[3,17,18] From these

studies it was concluded that many SWIs were caused by infection with the patient's own strain. These endogenous-infections could explain the findings in the current investigation.
Since PIs are associated with severe consequences it is important to identify risk factors which might be useful in prevention. Table 5 shows RR for several risk-factors for the development of PI. After logistic regression analysis only older age, CVO and OTH were independent, statistically significant risk factors. For the development of SWI only OTH was a significant risk factor. CVO frequently involves the implantation of a prosthetic valve. This explains the higher incidence of infections associated with CVO. The high incidence associated with OTH is probably because this group consisted mainly of extremely complicated procedures for congenital heart disease and dissecting aortic aneurysms. In the literature the use of IMA as a graft is frequently reported to be a risk factor for SWI.[14,15] This is especially true when both IMAs are used. In this study it was not recorded if one or both IMAs were used. Most of the IMA procedures in our institute however use only the left IMA as a graft. In this study, procedures involving IMA were not associated with a significantly increased RR compared with procedures using the SV. The rare use of both IMAs could be the explanation.
There is a trend towards operating older patients and performing more complicated procedures[8] (e.g. CVO and OTH) that may increase the incidence of PI. Therefore it will become increasingly important to develop new strategies to prevent these serious complications. The finding that cross-infection played a very limited role in the pathogenesis of SWI caused by *S. aureus*, may offer an opportunity for intervention. If endogenous infection plays a major role, elimination of nasal carriage should reduce the SWI rate. Further studies are warranted to evaluate the importance of nasal carriage for the development of SWI, and the effect of elimination of nasal carriage on the SWI rate.

*Acknoweledgemen*t: We thank C. Vandenbroucke-Grauls, and E. P. F. IJzerman for helpfull suggestions and critically reading the manuscript.

REFERENCES

1 *1991 Heart and Stroke Facts.* American Heart Association; 1991.
2 **Bucknall TE,** Factors affecting healing. In: T.E. Bucknall, H. Ellis Eds. *Wound healing for surgeons.* 1984;42.
3 **Calia FM, Wolinsky E, Motimer EA, Abrams JS, and Rammelkamp CH.** Importance of the carrier state as a source of *Staphylococcus aureus* in wound sepsis. *J Hyg Camb* 1969;**67**:49-57.
4 **Doebbeling BN, Pfaller MA, Kuhns KR, Massanari RM, Behrendt DM, and Wenzel RP.** Cardiovascular surgery profylaxis. A randomized, controlled comparison of cefazolin and cefuroxime. *J Thorac Cardiovasc Surg* 1990;**99**:981-9.
5 **Garner JS, Jarvis WR, Grace Emori T, Horan TC, and Hughes JM.** CDC definitions for nosocomial infections, 1988. *American J Inf Control* 1988;**16**:128-40.
6 **Green JW, Wenzel RP.** Postoperative wound infection: A controlled study of the increased duration of hospital stay and direct cost of hospitalization. *Ann Surg* 1977;**185**:264-8.
7 **Haley RW.** Surveillance by objective: a new priority-directed approach to the control of nosocomial infections. *Am J Inf Control* 1985;**13**:78-89.
8 **Jones EL, Weintraub WS, Craver JM, Guyton, RA, and Cohen CL.** Coronary bypass surgery: is the operation different today? *J Thorac Cardiovasc Surg* 1991;**101**:108-15.
9 **Kreter B, and Woods M.** Antibiotic prophylaxis for cardiothoracic operations. Metaanalysis of thirty years of clinical trials. *J Thorac Cardiovasc Surg.* 1992;**104**:590-9.
10 **Loop FD, Lytle BW, Cosgrove DM, *et al.*** Sternal wound complications after isolated coronary artery bypass grafting: early and late mortality, morbidity, and cost of care. *Ann Thor Surg* 1990;**49**:179-87.
11 **Nelson RM, and Dries DJ.** The economic implications of infection in cardiac surgery. *Ann Thor Surg* 1986;**42**:240-6.
12 SAS institute Inc. *SAS Users' Guide.* SAS institute INC, Gary, NC, 1989.
13 **Sellick JA, Stelmach M, and Mylotte JM.** Surveillance of surgical wound infections following open heart surgery. *Infect Control Hosp Epidemiol* 1991;**12**:591-6.
14 **Sethi GK, Copeland JG, Moritz T, Henderson W, Zadina K, and Goldman S** Comparison of postoperative complication between Saphenous Vein and IMA grafts to left anterior descending coronary artery. *Ann Thorac Surg* 1991;**51**:733-8.
15 **Ulicny KS, and Hiratzka LF.** The risk factors of median sternotomy infection: a current review. *J Cardiac Surg* 1991;**6**:338-51.
16 **Waldvogel F.** *Staphylococcus aureus* (including toxic shock syndrom). In: Mandell GL, Gordon Douglas R, and Bennet JE. Eds. *Principles and practice of infectious diseases.* 3rd edn. 1990;1489-510.
17 **Weinstein HJ** The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. *N Engl J Med* 1959;**260**:1303-8.
18 **Williams REO, Patricia Jevons M, Shooter RA *et al.*** Nasal staphylococci and sepsis in hospital patients. *Br Med J* 1959;**ii**:658-63.

CHAPTER ONE
SURGICAL WOUND INFECTIONS IN CARDIO-THORACIC SURGERY

PART TWO
NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* AS A MAJOR RISK FACTOR FOR WOUND INFECTIONS AFTER CARDIAC SURGERY

JAJW Kluytmans, JW Mouton, EPF IJzerman, CMJE Vandenbroucke-Grauls, AWPM Maat, JHT Wagenvoort and HA Verbrugh

Adapted from: *Journal of Infectious Diseases* 1995;**171**:216-9

postoperative stay in the intensive care unit; outcome (discharged alive or death); date of discharge or death; length of hospital stay; and readmittance (duration was added to the postoperative length of stay). Other postoperative infections were determined by CDC-criteria.[3] Site of infection, date of onset, and pathogens involved were recorded.
For cases, these additional variables were recorded: location of sternal wound infection (superficial or deep[3]), date of onset of infection, and the presence of secondary bacteremia.
Microbiology. All patients had nasal swabs the day before surgery. When a surgical wound infection was suspected, the wound and, if present, purulent discharge, were cultured. *S. aureus* was identified by standard microbiological methods. All available *S. aureus* isolates from preoperative nasal swabs and wounds were sent to the National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands) for phage typing.
Statistical analysis. Results were analyzed with the SAS statistical package.[9] Differences between groups were tested by Student's *t* test. The importance of the risk factors was determined by calculation of odds ratios and 95% confidence intervals (CIs). Odds ratios determined were Mantel-Haenszel estimates by a stratified analysis in which each stratum comprised a case and its controls. Statistical significance was accepted at $P<0.05$, two-tailed analysis.

RESULTS

Characteristics of patient population. In the study population 40 patients (2.0%) developed a sternal wound infection with *S. aureus*. A total of 120 patients were selected as controls. Table 1 shows the general characteristics of the cases and controls. No statistical significant differences were found in sex, body mass index (kg/m^2), type of surgery, and number of coronary arteries involved. The mean age for cases was significantly lower than for controls ($P=0.026$). The median postoperative length of stay was 30 days longer for cases than controls (Table 1). The total number of postoperative hospital days accrued by the 40 cases was 1901 days versus 1464 days for the 120 controls. Mortality was also significantly higher among cases ($P=0.014$). Four (10%) of the sternal wound infections were superficial and 36 (90%) were deep. Eighteen (45%) of the 40 sternal wound infections with *S. aureus* were associated with a secondary bacteremia. The median onset of sternal wound infections with *S. aureus* was 8 days after surgery. There was no significant difference in the incidence of postoperative infections other than sternal wound infections.
Analysis of risk factors. Table 2 shows the crude odds ratios with 95% CIs for the most important risk factors. Nasal carriage of *S. aureus* and insulin-dependent diabetes mellitus had the highest crude odds ratios. In addition to younger age (Table 1), these were the only statistically significant riskfactors. Two of the 4 insulin-dependent diabetes mellitus patients had *S. aureus* in their preoperative nasal cultures. To determine the minimum effect of nasal carriage, all 4 insulin-dependent diabetes mellitus patients were removed from the analysis. This analysis showed an odds ratio for nasal carriage of *S. aureus* of 8.8 with a 95% CI of 3.6-21.8. The variables not shown in table 2, were not associated with significant increased or decreased crude odds ratio.
Phage typing of *S. aureus* strains. Nineteen cases had positive preoperative *S. aureus* nasal cultures. For 10 of the 19, both pre- and postoperative isolates were available for phagetyping; these were identical for all 10. All *S. aureus* isolates were susceptible to oxacillin and aminoglycosides.

Table 1: *Characteristics of the patient population, types of surgical procedures performed, median postoperative length of stay and mortality in controls and cases.*

Variable	controls (n=120)	cases (n=40)
Mean age in years (SD+)	63.9 (9.0)‡	59.3 (11.5)‡
Gender (% females)	26.7	17.5
Mean body mass index (SD+)	25.4 (3.0)	25.9 (3.5)
Surgery:		
*CABG** without Internal Mammary Artery (%)	30.8	35.0
CABG*with Internal Mammary Artery (%)	52.5	54.2
Procedures involving cardiac valve replacement (%)	16.7	15.0
Other procedures†	2.5	7.5
Emergency procedures	5.8	10.0
Mean number of coronary arteries involved (SD+)	2.6 (1.3)	2.4 (1.1)
Median postoperative length of stay in days (range)	10 (6-50)‡	40 (9-145)‡
Mortality (%)	0.8‡	10.0‡

+ SD = Standard Deviation
* CABG = Procedure involving Coronary Artery Bypass Grafting
† Other Procedures are procedures other then CABG or cardiac valve replacement
‡ $P<0.05$

Table 2: *Crude Odds ratios and 95 % confidence intervals (CI) of the most important risk factors.*

Risk factor	number of events/number of observations (%)		Crude Odds ratio (CI)
	controls	cases	
Nasal carriage of *S. aureus*	15/120 (12.5)	19/36 (52.8)	9.6 (3.9-23.7)
Diabetes Mellitus: nonID+ & ID+	9/120 (7.5)	7/40 (17.5)	2.7 (0.9-8.0)
Diabetes Mellitus: nonID+	9/120 (7.5)	3/40 (7.5)	1.0
Diabetes Mellitus: ID+	0/120 (0)	4/40 (10.0)	21.0 (2.4-185.9)
Clindamycin as prophylaxis	11/120 (9.2)	0/40 (0)	0.4 (0.1-1.6)
Immunosuppressive drugs	10/120 (8.3)	6/40 (15.0)	1.9 (0.7-5.3)

+ ID is Insulin Dependent

DISCUSSION

Nasal carriage of *S. aureus*, insulin dependent-diabetes mellitus, and younger age were the most important and only statistical significant risk factors for development of sternal wound infection with *S. aureus*. Since patients with insulin-dependent diabetes mellitus have increased carriage rates of *S. aureus* and are probably at greater risk for developing *S. aureus* infections,[2] these two variables could have influenced each other. Because none of the controls had insulin-dependent diabetes mellitus, multiple logistic regression analysis could not be used to evaluate this interaction. The minimum effect of nasal carriage was therefore calculated by removing all insulin-dependent diabetes mellitus patients from the analysis. This analysis showed an odds ratio for nasal carriage of *S. aureus* of 8.8, which is only slightly different from the crude odds ratio of 9.6. Thus, we conclude that nasal carriage of *S. aureus* is an important and independent risk factor for development of sternal wound infections with *S. aureus.*

The importance of nasal carriage was confirmed by the 10 cases from whom pre- and postoperative isolates could be compared by phage typing. The pairs were all identical. The important role of endogenous infection also explains the heterogenous pattern of *S. aureus* isolates that caused surgical wound infections in our institution determined in a previous study.[6]

The nasal carriage rate of the entire 1980 patient study population can be approximated by extrapolation of the carriage rate of the controls and cases. By so doing, 264 (13.3%) of the 1980 patients would be nasal carriers, resulting in overall attack rates of 8.0% for carriers and 1.1% of noncarriers. This gives an attributable risk for nasal carriage of 86.3%.

Little attention has been paid to the role of nasal carriage in development of surgical wound infections for 25 years or so. In 1959, Williams *et al.* [13] reported that 2.1% of postoperative staphylococcal surgical wound infections occurred in 632 patients who never carried *S. aureus* in the nose. In 687 carriers, this incidence was 6.9% (Relative Risk [RR], 3.3; 95% CI, 1.8-6.1). In about half of the infected carriers, the wounds harbored staphylococci with the same phage type as that in the nose. Also in 1959, Weinstein *et al.* [12] analized nasal pathogens and postoperative infections in 125 patients undergoing major surgery. Those with positive preoperative nasal cultures (n = 43) had an infection rate of 37% versus 11% in 82 patients with negative preoperative cultures (RR, 3.4; CI, 1.6-7.0). In the nasal carriers, *S. aureus* was isolated from 94% of the infected wounds; a variety of organisms were cultured from the wounds of noncarriers. For nasal carriers with postoperative *S. aureus* infection, the phage types of the *S. aureus* isolated from the nose and wound were identical in 92% of cases. Similar results were reported by Calia *et al.*,[1] who calculated that about half of all surgical wound infections in their population were caused by endogenous infection with *S. aureus*. The surgical wound infection rate also correlated with the density of *S. aureus* in the nasal culture.

Several studies during the 1950s and 1960s attempted to eliminate nasal carriage preoperatively, but these attempts were hampered by a lack of effective alimination strategies. However, the recent introduction of mupirocin may offer new opportunities. This new topical antibiotic is highly effective for elimination of nasal carriage and is more effective than any other antibiotic studied to date.[2,4] Thus, it should be evaluated for its value in peri-operative prophylaxis.

Sternal wound infections with *S. aureus* were associated with a significant prolongation of the postoperative stay and a significantly higher mortality among cases. This high rate of severe infection is indicative of the unique pathogenicity of *S. aureus* in wound infections.[11] In a previous prospective surveillance study, we found that deep surgical wound infections in

thoracic surgery were associated with a median prolongation of postoperative stay of 30 days.[6] This is identical to the median prolongation of hospital stay in the present study. Although the degree of prolonged hospital stay and mortality rates vary in different studies, it is generally accepted that both are common consequences of sternal wound infections.[10]
Because of these severe consequences for patients and the high costs for the health care system, it is important to identify risk factors for development of sternotomy wound infections. To date, a multitude of patient- and procedure-related risk factors have been identified.[10] The current study differs from others in that it evaluates the risk factors for the development of sternal wound infections caused by one specific pathogen, *S. aureus*. Therefore, our results are not entirely comparable with those of studies that evaluated the consequences of and risk factors for development of sternal wound infections caused by a variety of pathogens.
In addition to nasal carriage, insulin-dependent diabetes mellitus and younger age were identified as risk factors for sternal wound infection with *S. aureus*. Insulin-dependent diabetes mellitus was also identified in other studies.[10] The risk factor of younger age seems paradoxical since, when age was identified as a risk factor in other studies, it was always older age.[10] One possible explanation is that some very complicated operations for congenital heart disorders were done on a small group of relatively young patients in this study. These procedures were associated with an increased risk for surgical wound infection in a previous study.[6]
Surgical procedures are becoming increasingly complicated, and the population of patients operated on has more underlying diseases.[5] This increases the risk for development of surgical wound infections. Therefore, new strategies are needed to keep the surgical wound infection rate and its associated morbidity and mortality as low as possible. Although the significance of nasal carriage of *S. aureus* for development of surgical wound infections has been well defined,[1,12,13] it has not been defined for cardiac surgery. Because of the great expense and morbidity and mortality associated with sternotomy wound infections, it is important to identify potential preventive measures. Further investigations are warranted to evaluate the effect of perioperative elimination of nasal carriage on surgical wound infection rates.

REFERENCES

1 **Calia FM, Wolinsky E, Motimer EA, Abrams JS, and Rammelkamp CH.** Importance of the carrier state as a source of *Staphylococcus aureus* in wound sepsis. *J Hyg Camb* 1969;**67**:49-57.

2 **Doebbeling BN, Breneman DL, Neu HC and the Mupirocin Collaborative Study Group.** Elimination of *Staphylococcus aureus* Nasal Carriage in Health Care Workers: Analysis of Six Clinical Trials with Calcium Mupirocin Ointment. *Clin Infect Dis* 1993;**17**:466-74.

3 **Garner JS, Jarvis WR, Grace Emori T, Horan TC, and Hughes JM.** CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 1988;**16**:128-40.

4 **Hudson IRB.** The efficacy of intranasal mupirocin in the prevention of staphylococcal infections: A review of recent experience. *J Hosp Infect* 1994;**27**:81-98.

5 **Jones EL, Weintraub WS, Craver JM, Guyton RA, and Cohen CL.** Coronary bypass surgery: is the operation different today? *J Thorac Cardiovasc Surg* 1991;**101**:108-15.

6 **Kluytmans JAJW, Mouton JW, Maat APWM, Manders MAAJ, Michel MF, and Wagenvoort JHT.** Surveillance of postoperative infections in thoracic surgery. *J Hosp Infect* 1994;**27**:139-47.

7 **Loop FD, Lytle BW, Cosgrove DM, *et al.*** Sternal wound complications after isolated coronary artery bypass grafting: early and late mortality, morbidity, and cost of care. *Ann Thor Surg* 1990;**49**:179-87.

8 **Nelson RM, and Dries DJ.** The economic implications of infection in cardiac surgery. *Ann Thor Surg* 1986;**42**:240-6.

9 SAS institute Inc. *SAS Users guide* SAS institute INC, Gary, NC, 1989.

10 **Ulicny KS, and Hiratzka LF.** The risk factors of median sternotomy infection: a current review. *J Cardiac Surg* 1991;**6**:338-51.

11 **Waldvogel F.** *Staphylococcus aureus* (including toxic shock syndrome). In: Mandell GL, Gordon Douglas R, and Bennet JE. Eds. Principles and practice of infectious diseases. *New York: Chuchill Livingstone, 3rd ed* 1990. 1489-510.

12 **Weinstein HJ.** The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. *New Engl J Med* 1959;**260**:1303-8.

13 **Williams REO, Patricia Jevons M, Shooter RA, *et al.*** Nasal staphylococci and sepsis in hospital patients. *Brit Med J* 1959;**ii**:658-63.

CHAPTER ONE
SURGICAL WOUND INFECTIONS IN CARDIO-THORACIC SURGERY

PART THREE
REDUCTION OF SURGICAL SITE INFECTIONS IN CARDIO-THORACIC SURGERY BY ELIMINATION OF NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*

JAJW Kluytmans, JW Mouton, MFQ VandenBergh, MAAJ Manders, APWM Maat, JHT Wagenvoort, MF Michel, and HA Verbrugh

Adapted from: *Infection Control Hospital Epidemiology* In press.

SUMMARY

Objective. To test the hypothesis that perioperative elimination of nasal carriage of *Staphylococcus aureus* using mupirocin nasal ointment reduces the surgical site infection (SSI) rate in cardio-thoracic surgery.
Design. Unblinded intervention trial with historical controls.
Setting. University Hospital, tertiary referral center for cardio-thoracic surgery.
Patients. Consecutive patients undergoing cardio-thoracic surgery between August 1, 1989 and February 1, 1991 (historical control group) and between March 1, 1991 and August, 1 1992 (intervention group). The intervention consisted of perioperative treatment with mupirocin nasal ointment (Bactroban nasal®).
Results. The historical control group consisted of 928 patients and the intervention group of 868, of whom 752 were actually treated. The 116 patients who were unintentionally not treated were considered as a concurrent control group. In the intention to treat analysis a significant reduction in SSI rate was observed after the intervention (historical control group 7.3 % and intervention group 2.8 %; $P<0.0001$). Also, the SSI-rate in the concurrent control group was significantly higher than in the treated group (7.8% and 2.0%, respectively; $P=0.0023$). Resistance of *S aureus* to mupirocin was not observed.
Conclusion. The results of this study indicate that perioperative elimination of nasal carriage using mupirocin nasal ointment significantly reduces the SSI rate in patients subjected to cardio-thoracic surgery and warrant a prospective, randomized, placebo-controlled study to determine the efficacy. This preventive measure may be beneficial in other categories of surgical patients as well.

INTRODUCTION

Cardio-thoracic surgery generally involves extensive procedures, which are associated with high rates of postoperative infections. These infections are associated with a significant prolongation of hospital stay [6,11,15,19] and mortality.[17] Based on the results of a previous study at our institute,[15] it was hypothesized that a substantial part of surgical site infections (SSI) might be caused by endogenous strains of *Staphylococcus aureus*. This hypothesis is supported by several studies dating back to the fifties and sixties which showed that nasal carriers of *S. aureus* had an increased risk for the development of SSI.[2,26,27] To evaluate the importance of nasal carriage as a risk factor for the development of sternal wound infection with *S. aureus* after sternotomy in our population, we have recently performed a case control study.[16] Preoperative nasal carriage was identified as the most important risk factor with an Odds ratio of 9.6 (95% confidence interval 3.9-23.7). Moreover, phage typing of both the pre- and post-operative isolates of *S. aureus* showed that all pairs (n=10) were identical. Based on these results we decided to evaluate whether elimination of nasal carriage reduces the SSI rate in patients undergoing cardio-thoracic surgery.
For eradication of nasal carriage, mupirocin calcium ointment was used because it has shown to be the most effective agent.[7,12,21]

PATIENTS AND METHODS

Population. The study population consisted of 1,796 patients undergoing cardio-thoracic surgery at the University Hospital Rotterdam, Dijkzigt. This is a tertiary referral center for cardiac and pulmonary surgery, and consists of an operating theater, a postoperative intensive care unit and a postoperative medical ward. The historical control group consisted of 928 and the intervention group of 868 consecutive patients. Patients who underwent more than one cardio-thoracic surgical procedure during the study period were included only the first time.
Perioperative procedures. The day before surgery all patients washed their body and hair with povidone-iodine soap or with chlorhexidine soap, hair removal was performed using depilatory creams, and a nasal swab was taken. Perioperatively, patients were given cefuroxime for 24 hours as systemic antibiotic prophylaxis. The loading dose (1.5 g) was administered one hour before the incision. Subsequent doses of 750 mg were administered every 6 hours. If patients reported an allergy to β-lactam-antibiotics, clindamycin (600 mg every 8 hours) was given instead. After surgery all patients stayed at the intensive care unit for at least 24 hours. These procedures were identical in the historical control and the intervention group.
Intervention. In the intervention group mupirocin calcium ointment (Bactroban nasal®, Smith-Kline Beecham, Pharmaceuticals, UK) was applied to each nostril, according to the manufacturers' instructions. The first dose was administered the day before surgery and the therapy was continued during five consecutive days, bid. Although it was the intention to treat all patients in the intervention group, not all patients were actually treated. The main reason why patients in the intervention group were not treated was unintentional omission by the nursing staff. This happened more often during the first months of the study. For comparisons the following groups were defined:

- Treated group: The patients in the intervention group who had actually been treated with mupirocin calcium ointment;
- Not treated group: The patients in the intervention group who had not received mupirocin calcium ointment. This group was evaluated as a concurrent control group.

Follow-up cultures were taken six to eight days after surgery in patients with *S aureus* in the preoperatively taken nasal cultures.

Surveillance method. During both periods the medical records were examined by one of the investigators for the presence of SSI using criteria as defined for this purpose by the Centers for Disease Control (CDC).[13] These definitions are based upon both clinical and laboratory parameters. Hospital identification number, sex, age, admission-, operation-, and discharge date from the department, in-hospital mortality, application of mupirocin, type of surgical procedure, surgeon and first assistant were recorded. Surgical procedures were divided into the following categories: (1) coronary artery bypass grafting (CABG) using saphenous veins (SV) as a graft only; (2) CABG using the internal mammary artery (IMA) as a graft; (3) cardiac valve replacement (CVR); (4) CVR combined with procedure 1; (5) CVR combined with procedure 2; (6) lung surgery and (7) other procedures. Lung operations were mainly performed for exploration and/or resection of malignancies. Surgical procedures which did not belong to either of the other categories accounted for the category of "other" procedures. This group consisted mainly of operations for congenital heart disorders and dissecting aneurysms of the aorta.

If SSI were present, the date of onset and the micro-organism(s) isolated were recorded. Surgical site infections were divided into incisional SSI (ISSI) and deep SSI (DSSI). The calculation of the incidence of SSI was based on the following principles: Only one SSI per patient was counted, and if a patient had both an ISSI and a DSSI at different sites, this patient was counted only as a DSSI.

Phage typing of *S. aureus* strains was performed at the National Institute of Public Health and Environmental Protection (RIVM, Bilthoven, The Netherlands). Mupirocin sensitivity was determined on all *S. aureus* strains isolated during the intervention period. The disk diffusion method was used as described previously.[8]

Statistical analysis. The results were analyzed using the SAS statistical package.[22] Categorical variables were tested by Chi square test or Fisher's exact test when appropriate. Continuous variables were tested by Student's *t* test.

RESULTS

The intervention group consisted of 868 patients and the historical control group of 928 patients. Of the 868 patients in the intervention group, 752 (86.6%) were actually treated. Table 1 shows the demographic variables of the intervention group, the historical control group, the treated group and the not treated group. Patients in the intervention group were slightly, but significantly younger (P=0.023). There was no significant difference in gender. In CABG procedures, the IMA was used more frequently in the intervention group compared to the historical control group (P=0.005). Also procedures involving CVR were significantly more frequent in the intervention group (P=0.008). There were no significant differences between the proportion of lung surgery or "other" procedures between the two groups. The treated and not treated group had comparable age distributions. There were significantly fewer males (P=0.008) and surgical procedures involving CVR (P<0.001) in the not treated group. The nasal carriage rates in the historical control group and in the treated group were comparable (15.1% and 16.0%, respectively). Follow-up cultures after application of mupirocin were available in 86 of the 120 patients with a preoperative nasal culture growing *S. aureus*. Eighty of the 86 (93%) cultures were negative.

The SSI rates of patients in the historical control, the treated and in the not treated group are shown in Table 2. Overall, the SSI-rate was 4.5% lower in the intention to treat group (2.8%) compared to the historical control group (7.3%, RR: 0.38, 95%CI: 0.24-0.60, P<0.0001). Furthermore, in the intervention group, the SSI rate was significantly lower in patients treated

(2.0%) compared to the not treated patients (7.8%, RR: 0.28, 95%CI: 0.12-0.57, P=0.0023). The SSI rate in the historical control group (7.5%) was comparable to that in the not treated group (7.8%, P>0.5).

Table 1: *Demographic variables of patients in the historical control, intervention, treated and not treated group.*

Variable	Historical control group	Intervention group	Treated group	Not treated group
Number of patients	928	868	752	116
Age				
mean	60.0	58.5	58.3	59.3
standard deviation	13.2	14.6	14.6	14.2
median	63.0	61.0	61.0	62.0
Sex				
male/female ratio	2.4	2.0	1.8	3.3
Surgical procedure				
CABG[+] using SV[*]	193 (20.8%)	122 (14.1%)	104 (13.8%)	18 (15.5%)
CABG[+] using IMA[†]	320 (34.5%)	319 (36.8%)	277 (36.8%)	42 (36.3%)
CVR[‡] (no CABG[+])	165 (17.8%)	194 (22.4%)	183 (24.3%)	11 (9.5%)
CVR[‡] and CABG[+] using SV[*]	37 (4.0%)	39 (4.5%)	33 (4.4%)	6 (5.2%)
CVR[‡] and CABG[+] using IMA[†]	19 (2.0%)	23 (2.6%)	23 (3.1%)	0 (0%)
Lung surgery	108 (11.6%)	82 (9.4%)	64 (8.5%)	18 (15.5%)
Other	86 (9.3%)	89 (10.3%)	68 (9.0%)	21 (18.1%)

[+] CABG: Coronary artery bypass graft
[*] SV: Saphenous vein
[†] IMA: Internal mammary artery
[‡] CVR: Cardiac valve replacement

Table 2 also shows the principal pathogens isolated from the infection sites. *S. aureus* and coagulase negative staphylococci (CNS) were evaluated as mupirocin susceptible micro-organisms. There was a highly significant reduction in the intervention group compared to the historical control group (RR: 0.44, 95%CI: 0.25-0.75, P=0.0032) as well as in the treated group compared to the not treated group (RR: 0.28, 95%CI: 0.11-0.75, P=0.0178). With regard to SSI caused by mupirocin resistant micro-organisms there also was a significant reduction in the intervention group compared to the historical control group (RR: 0.33, 95%CI: 0.12-0.91, P=0.0275). The reduction in the treated group compared to the not treated group was comparable but not statistically significant (RR: 0.23, 95%CI: 0.04-1.37, P=0.23).
The reduction of DSSI was more pronounced than that of ISSI when the intervention group was compared to the historical control group as well as when the treated group compared to the not treated group.
S. aureus was isolated from 9 of the 24 SSI (37.5%) in the intervention group and from 27 of the 68 SSI (39.7%) in the historical control group. All *S. aureus* isolates were susceptible to oxacillin and aminoglycosides. All *S. aureus* strains isolated from patients during the study period were susceptible to mupirocin. In the treated group, seven patients developed a SSI with *S. aureus*. The preoperative nasal cultures of these patients were all negative for *S. aureus*. Preoperative nasal cultures had not been performed in the patients who were unintentionally not treated. In the historical control group phagetyping was performed on 25 of 27 *S. aureus* strains isolated from SSI. Two pairs of identical strains were found. The other 21 strains had unique phage types. Comparison of the 9 *S. aureus* strains isolated from SSI in

the intention to treat group showed three pairs and three unique patterns (P=0.009, for proportion of pairs compared to the historical control group). Two of the three pairs consisted of one patient in the not treated group and one patient in the treated group. In both cases the not treated patient had developed the SSI before the patient in the treated group. Also the patients had been in the same ward during the same period. The third pair consisted of two patients in the treated group who had not been in the same ward during the same period. However, the same phagetype had been isolated recently from the nose of one of the nurses working on this ward.

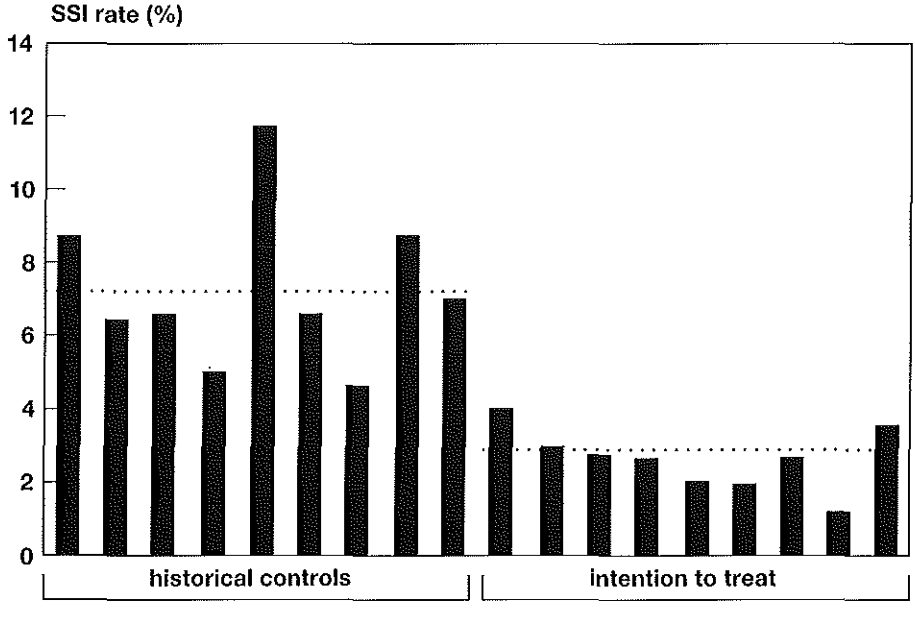

Figure 1: *Surgical wound infection rate per two-month-period in the historical control and intervention group.*

Table 2: *Number of surgical site infections (number/100 procedures) by type of infection,*

	Historical Controls (N=928)			Intervention group Treated (N=752)		
Principal pathogen	SSI	DSSI	ISSI	SSI	DSSI	ISSI
Mupirocin susceptible						
S. aureus	27(2.9)	18(1.9)	9 (1.0)	7 (0.9)	2 (0.3)	5 (0.7)
CNS[+]	14(1.5)	9 (1.0)	5 (0.5)	4 (0.5)	3 (0.4)	1 (0.1)
total	41(4.4)	27(2.9)	14(1.5)	11(1.4	5 (0.7)	6 (0.8)
Mupirocin resistant*	16(1.7)	7 (0.8)	9 (1.0)	3 (0.4)	1 (0.1)	2 (0.3)
No pathogen isolated	10(1.1)	3 (0.3)	7 (7.5)	1 (0.1)	0 (0)	1 (0.1)
Total	68(7.3)	37(4.0)	31(3.3)	15(2.0)	6 (0.8)	9 (1.2)

[+] CNS = coagulase negative staphylococci
* Mupirocin resistant includes aerobic gram-negative rods, *Pseudomonas aeruginosa*, and other isolates.

Figure 1 shows the SSI rate per two-month-period in the historical control and intervention group. It can be seen that there was not an increasing nor decreasing trend in both groups and that the effect of the intervention was apparant from the onset.

DISCUSSION

The reduction of the SSI rate in the intervention group compared to the historical control group was highly significant. This reduction was even greater in the group of patients who had been treated with mupirocin calcium ointment. In the group of patients who were unintentionally not treated the SSI rate was comparable to the historical control group. The reduction is, thus, most likely the result of the application of mupirocin.

Nasal carriage has been well established as a risk factor for SSI in several excellent studies a few decades ago.[2,26,27] Moreover, analysis of risk factors for the development of SSI on the department of cardio-thoracic surgery had recently identified nasal carriage as the most important independent variable.[16] In hemodialysis patients, it has also been shown consistently that nasal carriage of *S. aureus* is associated with an increased infection rate.[4,10,28] Mupirocin calcium ointment for eradication of carriage of in hemodialysis patients was highly effective, and resulted in a significant reduction of the *S. aureus* infection rate.[9,18] Therefore, it is likely that effective perioperative elimination of nasal carriage would also reduce the SSI rate. The efficacy of mupirocin to eliminate nasal carriage was 93%, which is comparable to the efficacy as reported previously.[7]

A major point of criticism is that in this study the comparison was made with a historical control group. This type of analysis can be biased by other unnoticed and, therefore, uncontrolled temporal changes which may impact upon the SSI-rate. Comparing the demographics of the historical control group and the intervention group there was a slightly, but significantly lower age in the intervention group. However, in a previous study we have found that lower age groups are at an increased risk for the development of sternal wound infection with *S. aureus*.[15] So this would have increased the SSI rate rather than reducing it. Surgical procedures in the intervention group included significantly more CVR procedures, in addition the IMA was used more frequently as a graft for CABG in this group of patients. In the historical control group these procedures were not associated with higher or lower SSI rates.[15] In several other studies, however, CVR and IMA have been identified as risk factors for the development of SSI.[23,25] If so, the observed increase in this types of surgical procedures would not have worked in favor of lower SSI rates in the intervention group.

Table 2: *principal pathogen and study group*

Intervention group		
Not Treated (N=116)		
SSI	DSSI	ISSI
2 (1.7)	2 (1.7)	0 (0)
4 (3.4)	2 (1.7)	2 (1.7)
6 (5.1)	4 (3.4)	2 (1.7)
2 (1.7)	1 (0.9)	1 (0.9)
1 (0.9)	1 (0.9)	0 (0)
9 (7.8)	6 (5.1)	3 (2.6)

There are several other arguments which support the conclusion that the observed reduction in SSI rates was due to the use of mupirocin. First, the difference in the SSI rates between the treated and the not treated patients in the intervention group provides supportive evidence for a beneficial effect of mupirocin. Secondly, it was found that the pathogenesis of *S. aureus* infections in the historical control group differed significantly from that in the intervention group. The proportion of *S. aureus* strains having a unique phagetype was significantly higher in the historical control group. This would fit in the concept that historical control patients were infected predominantly by their own, endogenous, strain of *S. aureus* and that the reduction of the SSI rate in the treated group was mainly due to a reduction of the number of endogenous acquired *S. aureus* infections. It is unlikely that other, unnoticed, factors would have such a selective effect on the source of the infecting strains. A third argument is that the reduction of the SSI rate was apparent immediately after the start of the intervention period. Prior to this moment, there was not a trend towards lower SSI rates in the historical control group, again supporting the conclusion that the highly significant reduction of the SSI rate was caused by the intervention. However, the finding that not only the SSI rate caused by mupirocin susceptible microorganisms was reduced but also the SSI rate caused by mupirocin resistant microorganisms provides an argument against the efficacy of mupirocin. The reasons for this observation can be that the observed effect was not caused by mupirocin but by other unnoticed temporal changes. However, this effect was not observed in the concurrent control group. Another explanation could be that nasal carriage of *S. aureus* is not always detected by a single culture due to limitations in sensitivity of this technique[20] or due to the effect of antibiotic usage in the days prior to cultures were obtained.
The application of mupirocin to the nares was well tolerated and side-effects were not observed. A point of concern is the development of resistance to mupirocin. Although not observed in this study, there have been several reports in the literature recently,[3,5,9,21] should be noted that almost all of these observations were related to inappropriate use.[14] This means use for longer than five days (sometimes for months), mostly on chronic skin infections. The risk for development of resistance with short perioperative, intranasal use is probably much lower. However, careful monitoring for development of resistance when using antibiotics is mandatory.
The results of this study indicate that perioperative elimination of nasal carriage significantly reduces the SSI rate in cardio-thoracic surgery. However, the results of our study do not justify the administration of mupirocin as perioperative prophylaxis to all patients undergoing cardio-thoracic surgery unless they are confirmed in a double-blind, randomized, placebo-controlled study. If it is confirmed that this method of prevention is effective in thoracic surgery, it may apply to other categories of surgical patients as well. Since *S. aureus* continuous to be the most important pathogen in SSI this new preventive strategy deserves to be evaluated further.

REFERENCES

1 **Boelaert JR, DeSmedt RA, De Baere YA, *et al.*** The influence of calcium mupirocin nasal ointment on the incidence of *Staphylococcus aureus* infections in hemodialysis patients. *Nephrol Dial Transplant* 1989;**4**:278-81.

2 **Calia FM, Wolinsky E, Mortimer EA, Abrams JS, and Rammelkamp CH.** Importance of the carrier state as a source of *Staphylococcus aureus in* wound sepsis. *J Hyg Camb* 1969;**67**:49-57.

3 **Chatfield CA, O'Neill WA, Cooke RPD, *et al.*** Mupirocin-resistant *Staphylococcus aureus* in a specialist school population. *J Hosp Infect* 1994;**26**:273-8.

4 **Chow JW and Yu VL.** *Staphylococcus aureus* nasal carriage in hemodialysis patients. *Arch Intern Med* 1989;**149**:1258-62.

5 **Cookson BD, Lacey RW, Noble WC, Reeves DS, Wise R and Redhead RJ.** Mupirocin-resistant *Staphylococcus aureus. Lancet* 1990;335:1095-6.

6 **Doebbeling BN, Pfaller MA, Kuhns KR, Massanari RM, Behrendt DM, and Wenzel RP.** Cardiovascular surgery prophylaxis. A randomized, controlled comparison of cefazolin and cefuroxime. *J Thorac Cardiovasc Surg* 1990;**99**:981-9.

7 **Doebbeling BN, Breneman DL, Neu HC and the Mupirocin Collaborative Study Group.** Elimination of *Staphylococcus aureus* nasal carriage in health care workers: Analysis of six clinical trials with calcium mupirocin ointment. *Clin Infect Dis* 1993;**17**:466-74.

8 **Fuchs PC, Jones RN and Barry AL.** Interpretive criteria for disk diffusion susceptibility testing of mupirocin, a topical antibiotic. *J Clin Microbiol* 1990;**28**:608-9.

9 **Gilbart J, Perry CR and Slocombe B.** High-level mupirocin resistance in *Staphylococcus aureus*: Evidence for two distinct isoleucyl-TRNA synthetases. *Antimicrob Agents Chemother* 1993;37:32-8.

10 **Goldblum SE, Reed WP, Ulrich JA, and Goldman RS.** Staphylococcal carriage and infections in hemodialysis patients. *Dial Transplant* 1978;**7**:1140-8.

11 **Green JW and Wenzel RP.** Postoperative wound infection: A controlled study of the increased duration of hospital stay and direct cost of hospitalization. *Ann Surg* 1977;**185**:264-8.

12 **Holton DL, Nicolle LE, Diley D and Bernstein K.** Efficacy of mupirocin nasal ointment in eradicating *Staphylococcus aureus* nasal carriage in chronic hemodialysis patients. *J Hosp Infect* 1991;**17**:133-7.

13 **Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Grace Emori T.** CDC definitions of nosocomial surgical site infections, 1992. *Infect Control Hosp Epidemiol* 1992;**13**:606-8.

14 **Hudson IRB.** The efficacy of intranasal mupirocin in the prevention of staphylococcal infections: A review of recent experience. *J Hosp Infect* 1994;**27**:81-98.

15 **Kluytmans JAJW, Mouton JW, Maat APWM, Manders MAAJ, Michel MF, and Wagenvoort JHT.** Surveillance of postoperative infections in thoracic surgery. *J Hosp Infect* 1994;**27**:139-47.

16 **Kluytmans JAJW, Mouton JW, IJzerman EPF, *et al.*** Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery. *J Infect Dis* 1995;**171**:216-9.

17 **Loop FD, Lytle BW, Cosgrove DM, *et al.*** Sternal wound complications after isolated coronary artery bypass grafting: early and late mortality, morbidity, and cost of care. *Ann Thor Surg* 1990;**49**:179-87.

18 **Manders MAAJ and Kluytmans JAJW.** Reduction of *Staphylococcus aureus* bacteremias by elimination of nasal carriage in hemodialysis. *Abstract nr 977.* 6th ECCMID, 1993, Seville, Spain.

19 **Nelson RM and Dries DJ.** The economic implications of infection in cardiac surgery. *Ann Thor Surg* 1986;**42**:240-6.

20 **Ogtrop ML.** Effect of broth enrichment cultures on ability to detect carriage of *Staphylococcus aureus. Antimicrobial Agents Chemother* 1996;39:2169.

21 **Reagan DR, Doebbeling BN, Pfaller MA, *et al.*** Elimination of coincident *Staphylococcus aureus* nasal and hand carriage with intranasal application of mupirocin calcium ointment. *Ann Intern Med* 1991;**114**:101-6.

22 SAS institute Inc. SAS Users guide SAS institute INC, Gary, NC, 1989.

23 **Sethi GK, Copeland JG, Moritz T, Henderson W, Zadina K, and Goldman S.** Comparison of postoperative complication between Saphenous Vein and IMA grafts to left anterior descending coronary artery. *Ann Thorac Surg* 1991;51:733-8.

SUMMARY

Objective. To assess the cost-effectiveness of perioperative intranasal application of mupirocin calcium ointment in cardio-thoracic surgery.
Design. Cost-effectiveness analysis, intervention study with historical controls.
Setting. University Hospital Rotterdam; tertiary referral center for cardiac and pulmonary surgery.
Patients. Consecutive patients undergoing cardio-thoracic surgery between 1 August 1989 and 1 February 1991 (control group, n=928), and between 1 March 1991 and 1 August 1992 (intervention group, n=868).
Intervention. Perioperative nasal application of mupirocin calcium ointment (Bactroban®, SmithKline Beecham Pharmaceuticals, United Kingdom), started on the day before surgery, continued for five days, twice daily.
Results. Postoperative costs were significantly increased in patients with a SSI, compared with uninfected patients (P<0.001). Mean SSI attributable costs were estimated at U$16,878 (95% CI: U$15,575 - U$18,181). The incidence of SSIs was 7.3% in the control group and 2.8% in the intervention group, mupirocin effectiveness being 62%. The costs of mupirocin were U$11 per patient. Thus, the savings per SSI prevented were U$16,633. To validate this comparative estimate of SSI attributable costs, a non-comparative analysis of the postoperative length of stay (POLS) was performed, according to the Appropriateness Evaluation Protocol. Approximately 50% of the comparative SSI attributable POLS was judged SSI attributable in the non-comparative analysis. Sensitivity analyses, testing for the robustness of our conclusions, indicated that the presented model is rather insensitive for variations in the incidence of SSIs and for the effectiveness and costs of mupirocin. SSI attributable costs were shown to be the only variable with substantial effect on the cost-effectiveness ratio. However, only when SSI attributable costs would be less than U$245, perioperative mupirocin would result in net costs instead of savings.
Conclusions. SSIs in patients undergoing cardio-thoracic surgery are associated with a substantial increase in postoperative costs. Provided that perioperative mupirocin reduces the SSI rate, this measure will be highly cost-effective in most centers providing cardio-thoracic surgical services.

INTRODUCTION

Surgical site infections (SSIs) constitute a substantial medical and socioeconomic problem, due to the prolonged and often severe morbidity resulting in an increased duration of hospitalization[1,3,5,8,12,15,18,19,25,29] and added hospital costs,[12,15,22,25,29] apart from the individual consequences for the affected patient. As nasal carriage of *Staphylococcus aureus* has been identified as an important risk factor for the development of a SSI,[2,20,34,35] elimination of nasal carriage might reduce the incidence of SSIs. Mupirocin calcium ointment has proven to be highly effective in the elimination of nasal carriage of *S. aureus*.[6,16,25]
Recently, an intervention study was performed to evaluate the effect of perioperative use of mupirocin nasal ointment on the incidence of SSIs and mortality in patients undergoing cardio-thoracic surgery.[21] The results indicated that perioperative elimination of nasal carriage using mupirocin nasal ointment significantly reduces the SSI rate in patients subjected to cardio-thoracic surgery. Although this finding could mean an important advance in the prevention of SSIs in cardio-thoracic surgery, concerns may be expressed about the potential costs of perioperative use of mupirocin in this large group of patients. In the present study we, therefore, performed a cost-effectiveness analysis of perioperative use of mupirocin nasal ointment in patients on cardio-thoracic surgery. The design of the intervention study, using a historical control group, has limitations with regard to its conclusions about the effectiveness of mupirocin. Likewise, the SSI rate and the SSI attributable cost will vary in different settings. Therefore, a sensitivity analysis of the cost-effectiveness was performed to test the robustness of the presented model to changes of input variables.

METHODS

Intervention study. The cost-effectiveness analysis was based on data derived from the intervention study mentioned above. An extensive description of the study population, intervention, perioperative procedures, and surveillance methods was published previously.[21]
In brief, the study population consisted of 1,796 consecutive patients undergoing cardio-thoracic surgery at the department of cardio-thoracic surgery of the University Hospital Rotterdam, the Netherlands, a tertiary referral center for cardiac and pulmonary surgery. Nine hundred and twenty-eight consecutive patients were operated between 1 August 1989 and 1 February 1991 and will be referred to as historical control group. Eight hundred and sixty-eight consecutive patients, operated between 1 March 1991 and 1 August 1992, will be referred to as intervention group.
In the intervention group mupirocin calcium ointment (Bactroban®, SmithKline Beecham, Pharmaceuticals, UK) was applied to each nostril, according to the manufacturer's instructions. The application was started on the day before surgery and was given twice daily for five days. Although it was the intention to treat all patients in the intervention group, 116 patients (14.4%) were not treated, mainly because of unintentional omission by the nursing staff.
Medical records were examined by one of the investigators for the presence of SSIs using criteria as defined for this purpose by the Centers for Disease Control (CDC).[10] Dates of admission, surgery, discharge and the time a patient stayed at various wards were derived from the Hospital Information System, as well as the number and type of laboratory-, radiodiagnostic-, and invasive procedures performed in each patient during the postoperative hospital stay.
Cost-effectiveness analysis. Costs of a five-day course of nasal mupirocin were U$11, as listed by the pharmaceutical company SmithKline Beecham, the Netherlands. Calculations of postoperative costs were performed in all patients from the historical control group. The

perspective taken in the calculation of costs was the hospital's point of view, i.e. hospital costs associated with the postoperative hospital stay were considered. Costs were estimated as the product of volume (number of hospital days, laboratory-, radiodiagnostic- and invasive procedures) and unit costs. Cost price studies were performed to determine unit costs reflecting the real use of resources. The year 1991 was used as a gauging year. In 1991 one US dollar corresponded with approximately 1.8 Dutch guilders. The cost of hospital days were estimated on the basis of the hospital cost accounting system. For each nursing unit the average direct and indirect costs of a hospital day have been calculated. Direct costs concerned manpower (nurses etc.) and materials (medical devices, medication, etc.), indirect costs were related to overheads. The costs per day ranged from U$265 for the medium care unit to U$930 for the postoperative intensive care unit. The postoperative length of stay (POLS) was defined as the number of postoperative hospital days, including the day of surgery and the day of discharge or death. In patients with a SSI, periods of readmission that were related to the infection were included in the POLS. The initial surgical procedure which could not have been influenced by the intervention with mupirocin was excluded from the analysis. The costs of laboratory procedures were also estimated on the basis of the hospital cost accounting system. For the radiodiagnostic- and invasive procedures the Dutch tariff system could be used as a close approximation of unit costs.

SSI attributable costs, i.e. additional postoperative costs for a patient with a SSI, were assessed by comparing costs in patients with and without a SSI. Since analyses comparing infected and uninfected patients are prone to bias due to differences (other than the SSI) between both groups that may influence the length and costs of hospital stay, a non-comparative method was applied to eliminate such bias. As approximately 80% of total postoperative costs were covered by costs of postoperative days (Table 1), the Appropriateness Evaluation Protocol, a non-comparative method which focuses on the POLS was used.[11] The method described in this protocol uses explicit criteria to determine the need for each day of hospital care, and enables the assessment of the SSI attributable POLS, i.e. that proportion of the total POLS that is attributable to the SSI. Medical records of 54 patients with a SSI (79%) were available for reviewing. Each day of hospital care was reviewed twice: once including all information related to symptoms and treatment of the SSI, and once excluding all this information. Days of hospitalization for which the first review indicated a need for hospital care, were considered appropriate. Appropriate days were attributed to the SSI if the second review of these days did not indicate a need for hospital care. A cost-effectiveness ratio, defined as costs per SSI prevented, was calculated.

Sensitivity analyses were developed to test a number of assumptions used in the construction of the model, including varying the incidence of SSIs from 1 to 100%; varying the effectiveness of mupirocin from 1 to 100%; varying the SSI attributable costs from 0 to 200% of the observed costs in patients with a SSI; and varying the costs of mupirocin treatment from U$0 to U$1000. The results from our previous intervention study[21] were used to provide an estimate of the SSI rate in clinical practice and an observed effectiveness of mupirocin.

Statistical analysis. Two-sided Student's *t* tests were used to test for differences in mean costs between groups, unless group numbers were smaller than 100 patients and the distribution of frequencies was skewed. In that case Mann-Whitney U - Wilcoxon rank sum tests were used.

RESULTS

The costs of the mupirocin treatment were U$11 per patient treated (one tube of 3 g, 0.2%, per patient). Costs associated with adverse events or the development of mupirocin resistance were zero, because neither one occurred. In Table 1 the costs of postoperative

procedures and postoperative hospital stay in the historical control group are presented. Both deep and incisional SSIs were associated with a statistically significant increase in postoperative costs when compared with patients without a SSI ($P<0.001$). Mean SSI attributable costs were U$16,878 (95% CI: U$15,575 - U$18,181). The incidence of SSIs in our intervention study decreased from 7.3% in the historical control group to 2.8% in the intervention group. Thus, mupirocin effectiveness was 62%, resulting in 45 SSIs prevented per 1000 patients undergoing surgery in the intervention group. From these observed figures the cost-effectiveness ratio was estimated at -U$16,633 per SSI prevented, i.e. U$16,633 were saved per SSI prevented.

Table 1: *Costs of postoperative procedures and hospital days in patients with a surgical site infection (SSI).*

		Costs (U$, 1991)			
Category	No. patients	Mean (SE)	SD	Median	p+
All patients	928				
Postoperative procedures		2,506 (151)	4,602	1,162	
Postoperative hospital days		5,580 (210)	6,402	4,236	
Total		8,086 (331)	10,071	5,439	
Incisional SSI	31				
Postoperative procedures		3,857 (830)	4,621	1,987	<0.001
Postoperative hospital days		8,668 (1,311)	7,300	7,000	<0.001
Total		12,525 (1,926)	10,722	8,998	<0.001
Deep SSI	37				
Postoperative procedures		10,443 (2,410)	14,660	6,935	<0.001
Postoperative hospital days		22,669 (3,596)	21,875	15,458	<0.001
Total		33,112 (5,462)	33,224	21,566	<0.001
Incisional or deep SSI	68				
Postoperative procedures		7,441 (1,414)	11,660	4,235	<0.001
Postoperative hospital days		16,286 (2,204)	18,175	9,132	<0.001
Total		23,727 (3,324)	27,409	14,560	<0.001
No SSI	860				
Postoperative procedures		2,116 (109)	3,190	1,096	
Postoperative hospital days		4,733 (100)	2,946	3,972	
Total		6,849 (187)	5,473	5,240	

+ versus no SSI

As the SSI attributable costs used in the calculation of this ratio may well be overestimated by using a comparative method, a non-comparative analysis was performed as well (vide supra). Table 2 shows the results of both the comparative and non-comparative analysis of the POLS. Based on the observed means of POLS in the comparative analysis, 21.4 days (95% CI: 14.0 - 28.8) were attributed to a SSI. In the non-comparative method, however, on average only 10.5 days (95% CI: 7.2 - 13.8) were considered to be due to the occurrence of an SSI. Likewise, the median number of days assigned to a SSI in the non-comparative analysis was only half the difference between the POLS medians of the two groups in the comparative analysis (7 days vs. 14 days).

The robustness of the presented model was tested by changing each input variable, while keeping the other variables at the baseline level. As indicated in Table 3 the cost-effectiveness ratio is relatively insensitive to variations in the effectiveness of mupirocin.

Table 2: *Postoperative length of hospital stay attributed to the surgical site infection (SSI) estimated from comparative and non-comparative analyses.*

Analysis	No. patients	Postoperative days		
		Mean (SE)	SD	Median
Comparative				
without SSI	860	13.4 (0.2)	7.0	11.0
with SSI	54	34.8 (3.7)	27.3	25.0
SSI attributable		21.4 (3.8)	-	14.0
Non-comparative				
Inappropriate	54	6.8 (1.1)	8.0	5.0
Appropriate, not SSI attributable	54	17.5 (3.3)	24.6	9.0
Appropriate, SSI attributable	54	10.5 (1.7)	12.6	7.0

Cost-effectiveness ratios vary from -U$16,727 to -U$15,371 per SSI prevented with mupirocin effectiveness ranging from 100% to 10%. Even with an effectiveness of mupirocin of only 1% the ratio is -U$1,809 per SSI prevented. Changes in the incidence of SSIs have even less impact; the ratio never exceeds -U$15,092 per SSI prevented and is never less than -U$16,860 per SSI prevented with incidence rates varying from 1 to 100%. Also, the costs of

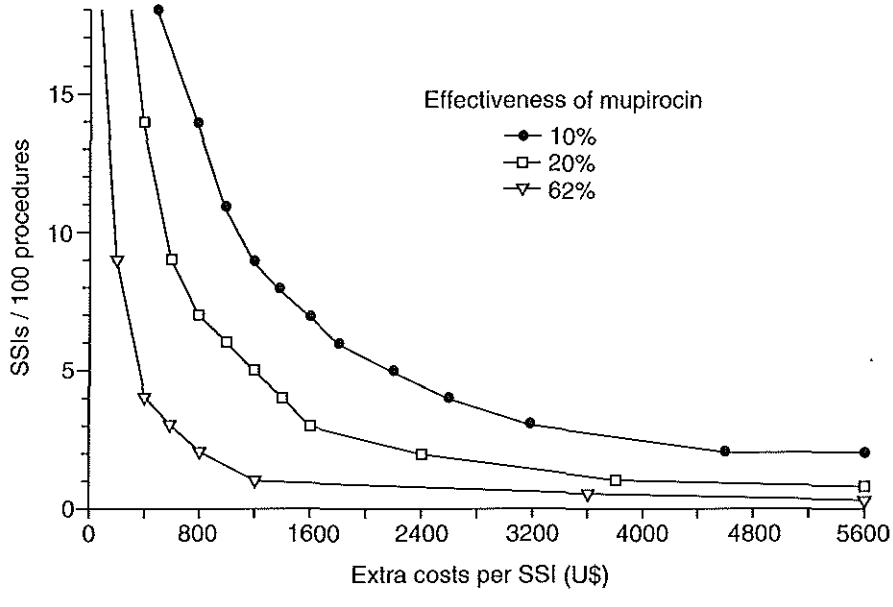

Figure 1. *Sensitivity analysis reporting cost-effectiveness ratios resulting when the surgical site infection (SSI) attributable costs and the incidence of SSIs are varied. The curved lines represent combinations of these variables that have a cost-effectiveness ratio of zero, at three different mupirocin effectiveness rates. Costs of mupirocin are U$11 per patient in all lines.*

mupirocin hardly influence the cost-effectiveness ratio; an increase in costs to U$759 per patient treated perioperatively would result in a ratio equalling zero. Changes in the SSI attributable costs, on the other hand, have a substantial effect on the cost-effectiveness ratio; a 50% increase in SSI attributable costs would result in a 50% decrease in the ratio (i.e. improves cost-effectiveness), while a 50% decrease in costs (e.g. using the non-comparative estimate of costs) would result in a 50% increase in the ratio (i.e. reduces cost-effectiveness). However, only when the SSI attributable costs would drop below U$245 per patient with a SSI, the cost-effectiveness ratio would exceed zero.
Figure 1 depicts the sensitivity of the cost-effectiveness ratio of perioperative mupirocin use to different assumptions regarding the incidence of SSIs without perioperative mupirocin use, the effectiveness of mupirocin, and the costs of SSIs. The curved lines represent the combinations of SSI attributable costs and SSI incidences that all have a cost-effectiveness ratio of zero for different mupirocin effectiveness rates. Even if both the incidence of SSIs and the effectiveness of mupirocin were reduced by 50%, SSI attributable costs should be less than U$978 per event to have a ratio of costs to SSI prevented above zero, i.e. not to be cost-effective.

Table 3: *Sensitivity analysis of the cost-effectiveness of perioperative nasal mupirocin in cardio-thoracic surgery.*

Scenario	Cost-effectiveness ratio (U$ per SSI prevented)*
Base case+	-16,633
Incidence of SSIs	
1%	-15,092
10%	-16,699
50%	-16,842
100%	-16,860
Effectiveness of mupirocin	
1%	-1,809
2%	-9,344
10%	-15,371
50%	-16,577
100%	-16,727
Mean SSI attributable costs	
U$0 (0%)	+244
U$245 (1.5%)	0
U$1,688 (10%)	-1,443
U$8,438 (50%)	-8,194
U$25,317 (150%)	-25,072
U$33,756 (200%)	-33,511
Costs of mupirocin (per patient)	
U$0	-16,878
U$50	-15,766
U$100	-14,654
U$250	-11,318
U$500	-5,759
U$759	0
U$1,000	+5,360

+ Incidence of SSIs: 7.3%; effectiveness of mupirocin: 62%; mean SSI attributable costs: U$16,878; and mupirocin costs: U$11 per patient (19).
* Figures <0 indicate savings per SSI prevented, i.e. perioperative mupirocin is cost-effective; figures >0 indicate net losses per SSI prevented, i.e. perioperative mupirocin is not cost-effective.

DISCUSSION

A recent report indicates that perioperative elimination of nasal carriage using mupirocin nasal ointment significantly reduces the incidence of SSIs in patients subjected to cardio-thoracic surgery.[21] Elimination of nasal carriage may become an important tool in the prevention of SSIs in the near future. However, concerns may arise about the potential costs of perioperative use of mupirocin. Our analysis indicates that perioperative use of mupirocin nasal ointment in patients on cardio-thoracic surgery is highly cost-effective, with a cost-effectiveness ratio of -U$16,633 per SSI prevented, i.e. U$16,633 saved per SSI prevented. Estimates of costs and effects in our analysis were partly based on a number of assumptions. First, the 7.3% incidence of SSIs was derived from a recently performed intervention study, as mentioned before.[21] As incidence rates of SSIs differ between hospitals and between countries,[35] cost-effectiveness ratios of perioperative use of mupirocin may vary too in different settings. Also, the effectiveness of perioperative mupirocin, which may influence the cost-effectiveness ratio calculated, was based on the results of the intervention study. The design of this study, using a historical control group, could have biased the results. Unnoticed and, therefore, uncontrolled temporal changes other than the intervention with mupirocin, may have influenced the SSI rate. However, as reported previously,[21] the incidence of SSIs in the intervention group was significantly lower among those patients that actually received mupirocin than in those patients that were unintentionally not treated. Moreover, the reduction in SSI rate was apparent immediately after the start of the intervention, while no trend towards lower SSI rates was discernible before the intervention was implemented.
Thirdly, the costs of a five-day course of nasal mupirocin (one tube of 3 g, per patient) were U$11 as listed by the pharmaceutical company in the Netherlands. In other countries, the costs of mupirocin might be different. In our study all patients undergoing cardio-thoracic surgery were targeted for perioperative nasal mupirocin. Considering the increasing risk for the development of mupirocin resistance with large scale perioperative mupirocin use, one might argue to restrict perioperative nasal mupirocin to those patients who have *S. aureus* isolated from a preoperative nasal culture. Assuming a maximum nasal carriage rate of 50%, the costs of the intervention would increase to U$26.5 per patient undergoing surgery (nasal culture: U$21; mupirocin: U$5.5). As shown by our sensitivity analysis, however, such an increase in costs will have little influence on the cost-effectiveness ratio of the intervention.
Finally, the calculation of SSI attributable costs may have influenced our estimate of the cost-effectiveness ratio. Costs were based on a cost profile and tariff system which is specific for the Netherlands. In other cost- and reimbursement systems, SSI attributable costs may be quite different, and therewith the cost-effectiveness of perioperative mupirocin. A very important factor in the assessment of SSI attributable costs is the method used to determine which proportion of total costs are SSI attributable. Although several studies have clearly shown that the development of a SSI is associated with an increase in the duration of hospitalization[1,3,5,8,12,15,18,19,25,29] and associated costs,[12,15,22,25,29] the methods employed in order to estimate the added hospital stay and costs in nosocomial infections (NIs) vary considerably.[14] As a result, there are discordant findings depending upon the particular method used.[4,31] A frequently used method is the direct comparison, in which the additional hospital stay or costs are assessed by comparing the length of stay of infected and uninfected patients.[1,3,5,8,12,14,15,18,19,22,25] Either unmatched[3,9,14,22] or matched [1,8,12,18,25,29] control patients can be used. A fundamental limitation with the unmatched group comparison method is the implicit assumption that any difference in length of stay or costs is attributable to the NI, and not related to other inherent differences between those patients who do and those who do not acquire a NI (e.g. age, surgical procedure, severity of illness, and comorbidities). Studies using matched controls have adjusted for some, but not all, of these known riskfactors and do not control for riskfactors that are yet unknown.[1,13,14,25,29,33] The matched

control estimates of NI attributable days have been lower than in the unmatched group approach,[4,31] however, they may still overestimate the incremental days.[14,23] In contrast, the direct-attribution approaches develop estimates of infection attributable days and costs by focusing only on the cases with an infection, thus eliminating problems associated with differences between cases and controls. Direct-attribution analyses include the clinician judgement approach [4,7,9,14,15,17,31] and the standardized case review approach.[31-33] The Appropriateness Evaluation Protocol (AEP) is a standardized case review method, in which standardized and objective criteria are used to determine which days of stay should be attributed to the NI versus those attributable to other health problems.[11] Under the assumption that all pertinent clinical information justifying use of inpatient services is contained in the medical record, and that a distinction can be made between information related to the original causes for hospitalization and information related to the NI, the AEP is known to be a reliable and valid instrument in the assessment of the NI related hospital stay. It supplies conservative estimates.[33,27,28,30] Our non-comparative analysis of the POLS did indeed yield much less SSI attributable days than the comparative analysis did. However, even if this conservative estimate of POLS is used to calculate the SSI attributable costs, perioperative use of mupirocin would still be cost-effective.

The effectiveness of mupirocin has not been definitely established, and awaits the results of a double blind placebo controlled randomized trial. However, even with an effectiveness of only 1%, our sensitivity analysis revealed that perioperative administration of nasal mupirocin will be a cost-effective measure. We, therefore, conclude that, provided that perioperative mupirocin reduces the SSI rate, this measure will be highly cost-effective in most groups of patients undergoing cardio-thoracic surgery. Restricting perioperative use of nasal mupirocin to nasal carriers of *S. aureus* has negligible effect on the cost-effectiveness and may, therefore, be the best prophylactic strategy that will both be cost-effective and prevent the emergence of mupirocin resistance among *S. aureus*.

REFERENCES

1 **Boyce JM, Potter-Bynoe G, Dziobek L.** Hospital reimbursement patterns among patients with surgical wound infections following open heart surgery. *Infect Control Hosp Epidemiol* 1990;**11**:89-93.

2 **Calia FM, Wolinsky E, Mortimer EA Jr., Abrams JS, Rammelkamp CH.** Importance of the carrier state as a source of *Staphylococcus aureus* in wound sepsis. *J Hyg Camb* 1969;**67**:49-57.

3 **Clarke SKR.** Sepsis in surgical wounds with particular reference to *Staphylococcus aureus*. *Br J Surg* 1957;**44**:592-6.

4 **Daschner F.** Cost-effectiveness on hospital infection control - lessons from the 1990s. *J Hosp Infection* 1989;**13**:325-36.

5 **Doebbeling BN, Pfaller MA, Kuhns KR, Massanari RM, Behrendt DM, Wenzel RP.** Cardiovascular surgery prophylaxis. A randomized, controlled comparison of cefazolin and cefuroxime. *J Thorac Cardiovasc Surg* 1990;**99**:981-9.

6 **Doebbeling BN, Breneman DL, Neu HC, Aly R, Yangco BG, Holley HP Jr. Marsh RJ, Pfaller MA, McGowan JE Jr. Scully BE, Reagan DR, Wenzel RP, Mupirocin Collaborative Study Group.** Elimination of *Staphylococcus aureus* nasal carriage in health care workers: analysis of six clinical trials with calcium mupirocin ointment. *Clin Infect Dis* 1993;**17**:466-74.

7 **Finkler SA.** The distinction between costs and charges. *Ann Intern Med* 1982;**96**:102-9.

8 **Freeman J, Rosner BA, McGowan JE Jr.** Adverse effects of nosocomial infections. *J Infect Dis* 1979;**140**:732-40.

9 **Freeman J, McGowan JE Jr.** Methodological issues in hospital epidemiology. III. Investigating the modifying effects of time and severity of underlying illness on estimates of cost of nosocomial infection. *Rev Infect Dis* 1984;**6**:285-300.

10 **Garner JS, Jarvis WR, Grace Emori T, Horan TC, Hughes JM.** CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 1988;**16**:128-40.

11 **Gertman PM, Restuccia JD.** The Appropriateness Evaluation Protocol: a technique for assessing unnecessary days of hospital care. *Med Care* 1981;**19**:855-71.

12 **Green JW, Wenzel RP.** Postoperative wound infection: a controlled study of the increased duration of hospital stay and direct cost of hospitalization. *Ann Surg* 1977;**185**:264-8.

13 **Green MS, Rubinstein E, Amit P.** Estimating the effects of nosocomial infections on the length of hospitalization. *J Infect Dis* 1982;**145**:667-72.

14 **Haley WH, Schaberg DR, Von Allmen SD, McGowan JE Jr.** Estimating the extra charges and prolongation of hospitalization due to nosocomial infections: a comparison of methods. *J Infect Dis* 1980;**141**:248-57.

15 **Haley RW, Schaberg DR, Crossley KB, von Allmen SD, McGowan JE Jr.** Extra charges and prolongation of stay attributable to nosocomial infections: a prospective interhospital comparison. *Am J Med* 1981;**70**:51-8.

16 **Holton DL, Nicolle LE, Diley D, Bernstein K.** Efficacy of mupirocin nasal ointment in eradicating *Staphylococcus aureus* nasal carriage in chronic hemodialysis patients. *J Hosp Infect* 1991;**17**:133-7.

17 **Kaiser AB, Petracek MR, Lea JW, et al.** Efficacy of cefazolin, cefamonadole, and gentamicin as prophylactic agents in cardiac surgery. *Ann Surg* 1987;**206**:791-7.

18 **Kappstein I, Schulgen G, Fraedrich G, Schlosser V, Schumacher M, Daschner FD.** Added hospital stay due to wound infections following cardiac surgery. *Thor Cardiovasc Surgeon* 1992;**40**:148-51.

19 **Kluytmans JAJW, Mouton JW, Maat APWM, Manders MAAJ, Michel MF, Wagenvoort JHT.** Surveillance of postoperative infections in thoracic surgery. *J Hosp Infect* 1994;**27**:139-47.

20 **Kluytmans JAJW, Mouton JW, IJzerman EPF, Vandenbroucke-Grauls CMJE, Maat AWPM, Wagenvoort JHT, Verbrugh HA.** Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery. *J Infect Dis* 1995;**171**:216-9.

21 **Kluytmans JAJW, Mouton JW, VandenBergh MFQ, Manders MAAJ, Maat APWM, Wagenvoort JHT, Michel MF, Verbrugh HA.** Reduction of surgical wound infections in cardio-thoracic surgery by elimination of nasal carriage of *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* In press.

22 **Loop FD, Lytle BW, Cosgrove DM, Mahfood S, McHenry MC, Goormastic M, Stewart RW, Golding LAR, Taylor PC.** Sternal wound complications after isolated coronary artery bypass grafting: early and late mortality, morbidity, and cost of care. *Ann Thorac Surg* 1990;**49**:179-87.

23 **McGowan JE Jr.** Cost and benefit in control of nosocomial infection: methods for analysis. *Rev Infect Dis* 1981;**3**:790-7.

24 **McGowan JE.** Cost and benefit - a critical issue for hospital infection control. *Am J Infect Control* 1982;**10**:100-8.

25 **Nelson RM, Dries DJ.** The economic implications of infection in cardiac surgery. *Ann Thor Surg* 1986;**42**:240-6.

26 **Reagan DR, Doebbeling BN, Pfaller MA, Sheetz CT, Houston AK, Hollis RJ, Wenzel RP.** Elimination of coincident *Staphylococcus aureus* nasal and handcarriage with intranasal application of mupirocin calcium ointment. *Ann Int Med* 1991;**114**:101-6.

27 **Restuccia JD, Gertman PM, Dayno SJ, et al.** A comparative analysis of appropriateness of hospital use. *Health Aff* 1984;**3**:130.

28 **Rishpon S. Lubacsh S, Epstein LM.** Reliability of a method of determining the necessity for hospitalization days in Israel. *Med Care* 1986;**24**:279-82.

29 **Rubinstein E, Green M, Modan M, Amit P.** The effects of nosocomial infections on the length and costs of hospital stay. *J Antimicrob Chemother* 1982;**9, Suppl A**:93-100.

30 **Stevenson RC, Blackmann SC, Willimas CL, Bartzokas CA.** Measuring the saving attributable to an antibiotic prescribing policy. *J Hosp Infect* 1988;**11**:16-25.

31 **Wakefield DS, Pfaller MA, Hammons GT, Massanari RM.** Use of the appropriateness evaluation protocol for estimating the incremental costs associated with nosocomial infections. *Med Care* 1987;**25**:481-8.

32 **Wakefield DS, Helms CM, Massanari RM, Motomi M, Pfaller M.** Cost of nosocomial infection: relative contribution of laboratory, antibiotic, and per diem costs in serious *Staphylococcus aureus* infections. *Am J Infect Control* 1988;**16**:185-92.

33 **Wakefield DS, Pfaller M, Ludke RL, Wenzel RP.** Methods for estimating days of hospitalization due to nosocomial infections. *Med Care* 1992;**30**:373-6.

34 **Weinstein HJ.** The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. *N Engl J Med* 1959;**260**:1303-8.

35 **Williams REO, Jevons MP, Shooter RA, Hunter CJW, Girling JA, Griffiths JD, Taylor GW.** Nasal staphylococci and sepsis in hospital patients. *Br Med J* 1959;**ii**:658-63.

36 **Wong ES.** Surgical site infections. In: Mayhall CG, editor. Hospital epidemiology and infection control. *Baltimore: Williams and Wilkins*, 1996:154-75.

CHAPTER TWO
BACTEREMIA IN PATIENTS ON HEMODIALYSIS

PART ONE
PREVENTION OF *STAPHYLOCOCCUS AUREUS* BACTEREMIA IN HEMODIALYSIS PATIENTS BY ELIMINATION OF NASAL CARRIAGE USING MUPIROCIN

Jan Kluytmans, Marie-Jose Manders, Erik van Bommel, and Henri Verbrugh

Adapted from: *Infect Control Hosp Epidemiol* In press.

SUMMARY

Objective. To study the efficacy of mupirocin for the elimination of nasal carriage of *Staphylococcus aureus* in hemodialysis patients and its effect on the incidence of *S. aureus* bacteremia.

Design. The efficacy of mupirocin was studied in a prospectively followed cohort. The effect of this intervention on the rate of *S. aureus* bacteremia was evaluated using a historic control group.

Setting. Patients on the hemodialysis unit of the University Hospital Rotterdam, a tertiary referral center.

Patients. The study group consisted of consecutive patients on hemodialysis from 1 February, 1992, until 1 November, 1993. They were screened by taking nasal cultures monthly during there time on hemodialysis. If *S. aureus* was isolated, treatment with mupirocin nasal ointment was initiated. The control group consisted of patients treated on the same hemodialysis unit from 1 January, 1990 untill 1 January, 1992.

Results. The study group consisted of 226 patients, of which 172 were evaluable to determine the efficacy of mupirocin. Sixty-seven (39%) were identified as nasal carriers. Immediately after the initial treatment, 66 (98.5%) nasal cultures became negative. After 3 months and 6 months, respectively, 63 (94%) and 61 (91%) of the treated carriers had negative cultures. The rate of bacteremia, being the number of episodes of *S. aureus* bacteremia per patient year on hemodialysis, in the 226 patients in the study group was significantly lower compared with this rate in the 273 patients in the control group (respectively 0.04 and 0.25, $P<0.001$). Development of resistance and adverse effects were not observed.

Conclusions. Mupirocin nasal ointment effectively eliminates nasal carriage of *S. aureus* in patients on hemodialysis, thereby significantly reducing the incidence of *S. aureus* bacteremia.

INTRODUCTION

Infection is the most common cause of morbidity and the second cause of mortality in patients on hemodialysis,[21] *Staphylococcus aureus* being the most frequently isolated pathogen.[5,13] Nasal carriage of *S. aureus* plays an important role in the pathogenesis of these infections.[7,11,13] Expressed longitudinally, approximately 30% of the normal population will be prolonged and 50% will be intermittent carriers, whereas 20% will never be colonized.[9] The underlying mechanisms for these differences are presently unknown. The adherence of *S. aureus* to the nasal epithelial cells seems to be mediated by the lipoteichoic acid moiety in the cell wall.[1] Increased rates of nasal carriage are found among groups of patients in which the skin is often punctured, e.g. by needles or intravascular devices. This is especially the case in patients with insulin dependent diabetes mellitus,[3,18] patients on chronic hemodialysis [3,14] and intravenous drug addicts.[3,17]

Nasal carriage of *S. aureus* has been associated with an increased risk of infection in patients after surgery,[19] in patients on continuous ambulatory peritoneal dialysis (CAPD)[15] and in patients on hemodialysis.[7,11,21] Besides their increased colonization by *S. aureus*, patients on chronic hemodialysis are more prone to infections with *S. aureus* because of their suppressed immunity and the presence of a vascular access site.[7]

In hemodialysis patients, several studies have shown that elimination of nasal carriage reduces the incidence of *S. aureus* infections.[4,12,21] In a study by Yu *et al.*,[21] nasal carriage was effectively eliminated by oral administration of rifampin. This resulted in a significant decrease of infections with *S. aureus*. However, development of resistance to rifampin was observed more than once during this study. This limits the use of rifampin for this purpose. Another approach to eliminate nasal carriage is the local application of antibiotics to the nose. Topical gentamicin, vancomycin and bacitracin have been studied for this purpose. The elimination of nasal carriage was not effective, being shorter than two weeks for all regimens.[7] Mupirocin, a topical antibiotic, represents a new class of antibiotics. Unlike any other known antimicrobial agent, it inhibits the bacterial isoleucyl TRNA synthetase, thereby blocking protein synthesis and indirectly inhibiting RNA synthesis. Mupirocin has an excellent activity against *S. aureus*, the minimal inhibitory concentrations being ≤ 1 mg/l. The nasal ointment has proven to be safe and highly effective in eradication of nasal carriage of *S. aureus* in carriers without severe underlying diseases.[6,8,16]

To determine the efficacy of elimination of nasal carriage using mupirocin and its effects on the incidence of *S. aureus* bacteremia in the patients on hemodialysis in our hospital, an intervention study was initiated.

METHODS

Study group. From 1 February, 1992 until 1 November, 1993, all patients on the hemodialysis unit of the University Hospital Rotterdam, were routinely screened for nasal carriage of *S. aureus* by taking nasal cultures monthly during their time on hemodialysis. Patients with positive nasal cultures for *S. aureus* were treated initially with mupirocin nasal ointment twice a day for 5 days. Two days later a nasal culture was taken to determine the immediate efficacy. After the initial treatment, mupirocin was applied once weekly. Patients with consistent negative nasal cultures after an initial positive culture were considered cured. If the nasal culture became positive again, this was considered as a treatment failure. For all patients age, gender, the total number of days on hemodialysis, the number of episodes of *S. aureus* bacteremia and the results of nasal cultures were recorded. Patients from whom no nasal culture was performed or who were colonized by methicillin or tobramycin resistant *S. aureus*, were excluded from the evaluation of the efficacy of elimination of nasal carriage. For

comparison of the incidence of *S. aureus* bacteremia, all patients were included for an intention to treat analysis. The treated and not treated patients in the study group were also evaluated separately.

The following groups were defined:

- Intention to treat group: All patients on hemodialysis during the study period;
- Treated group: Patients on hemodialysis during the study period, from whom nasal cultures were obtained and if positive were treated with mupirocin;
- Untreated group: Patients who were excluded from the study group.

Control group. All patients of the hemodialysis unit at our hospital between 1 January, 1990 and 1 January, 1992 were used as an historic control group. During this period nasal swabs were not taken routinely, and no efforts were made to eliminate nasal carriage. For all control patients age; sex; the total number of days on hemodialysis; and the number of episodes of *S. aureus* bacteremias were recorded.

Microbiology. Nasal cultures were obtained using a pre-moistened dacron swab, which was applied to both anterior nasal vestibules. The swabs were streaked on columbia agar base (supplemented with 5% sheep blood) and on mannitol salt agar plates, which were incubated for at least 48 hours at 37°C, aerobically. The plates were examined for the presence of *S. aureus*, of which sensitivity patterns were determined, including methicillin, tobramycin and mupirocin. The Kirby-Bauer agar disc method was used.

Statistical analysis. The differences between groups were evaluated by the Chi square test or the *t* test. Significance was accepted when $P<0.05$.

RESULTS

Patient characteristics. Hemodialysis was performed on 226 patients during the study period and on 273 patients during the control period. The male/female ratio and age characteristics of both groups are shown in Table 1. There were no significant differences between the groups.

Table 1: *Patient characteristics in the study and the control group.*

variable	control group	study group
number of patients	273	172
number of females (%)	105 (38.5)	64 (37.2)
mean age (range)	54.1 (19-85)	55.8 (17-87)
median age	57	55

Nasal carriage of *S. aureus*. Fifty-four patients with a total of 3.8 year on hemodialysis could not be evaluated for the efficacy of elimination of nasal carriage, and were excluded from the study group. The reason for exclusion are shown in Table 2. This leaves 172 patients who were evaluable for nasal carriage and efficacy of mupirocin nasal ointment. In the study group 67 (39.0%) of a total of 172 patients were identified as nasal carriers. After the initial treatment with mupirocin of 5 days, 66 (98.5%) nasal cultures became negative. After 3 months and 6 months, respectively, 63 (94%) and 61 (91%) of the treated carriers had negative cultures.

Table 2: *Reasons for exclusion from the study group.*

reason for exclusion	number of patients
no initial nasal culture performed	47
no control nasal cultures taken after treatment	5
aminoglycoside resistant *S. aureus* isolated	2
total	54

***S. aureus* bacteremia.** In the study group, 4 episodes of *S. aureus* bacteremia occurred in 226 patients with a total of 100.3 years on hemodialysis. This gives an incidence of 0.04 bacteremia/patient year on hemodialysis in the intention to treat group. One of these bacteremias occurred in a patient who was excluded from the study group, and had subsequently not received mupirocin. In this group of untreated patients the incidence was 0.25 bacteremia/patient year on hemodialysis. In the 172 patients in the study group who were treated, 3 episodes of *S. aureus* bacteremia occurred, giving an incidence of 0.03 bacteremia/patient year on hemodialysis. In the control group there were 273 patients with a total of 100.0 years on hemodialysis. Twenty-five episodes of *S. aureus* bacteremia were recorded. This gives an incidence of 0.25 *S. aureus* bacteremia/patient year on hemodialysis. The reduction of the incidence of *S. aureus* bacteremia in the intention to treat group and in the treated group compared to the historic control group was statistically significant. The incidences of bacteremia in various groups are summarized in Table 3.

Table 3: *Incidence of episodes of S. aureus bacteremia in the control, the intention to treat, the treated and the not treated group.*

Group	n	Total years on hemodialysis	Number of episodes of *S. aureus* bacteremia	Year-incidence of *S. aureus* bacteremia	*P*-value compared to control group
Control	273	100.0	25	0.25	
Intention to treat	226	100.3	4	0.04	<0.001
Treated	172	96.5	3	0.03	<0.001
Not treated	54	3.8	1	0.25	>0.5

Susceptibility of *S. aureus*. All *S. aureus* strains were susceptible to mupirocin. Also all strains were susceptible to methicillin and all but two were susceptible to tobramycin.
Adverse events. No side effects occurred during the study period and mupirocin nasal ointment was well tolerated.

DISCUSSION

In the study group the rate of nasal carriage was 39.0%, which is comparable to the rates reported in other studies.[4,7] The efficacy of the elimination of nasal carriage immediately after the initial 5 day treatment course was high (98.5%). After 6 months 91.0% of the nasal cultures were still negative. The effect of the intervention on the incidence of infectious complications with *S. aureus* was measured by comparing the rates of bacteremic episodes with *S. aureus* before and after the intervention. The rate of bacteremia with *S. aureus* was chosen because it is an objective parameter, which was well documented in both the study group and the control group. The reduction of the rate of bacteremia in the intention to treat group as compared to the historic control group was sixfold. This was highly significant. Since an intervention study with a historical control group can be biassed by other, unnoticed

changes the interpretations in this study should be made carefully. However, it has been shown consistently that effective elimination of nasal carriage in patients on hemodialysis lowers the infection rate with *S. aureus*.[4,12,21] The elimination of nasal carriage in this study was highly effective, in fact more effective than with any other treatment which has been studied. It is therefore unlikely that the reduction of the rate of *S. aureus* bacteremia was caused by other factors. Moreover, in a prospective, randomised, double-blind, placebo-controlled study, Boelaert *et al.*[4] have shown that mupirocin nasal ointment was effective in hemodialysis patients. The nasal carriage rate in the treated group was significantly lower than in the placebo group. Also the incidence of *S. aureus* infections was significantly lower in the treated group. Development of mupirocin resistance among *S. aureus* strains was not observed during this study. Therefore we conclude that application of mupirocin nasal ointment to hemodialysis patients who are nasal carriers of *S. aureus* effectively eliminates nasal carriage and thereby significantly reduces the rate of bacteremia with *S. aureus*.

Although not observed in this study, a matter of concern is the increasing number of observations addressing the development of resistance to mupirocin. This resistance consists of an intermediate level form (MIC 8-256). This form is clinically less important and depends on modifications in the bacterial isoleucyl-TRNA synthetase. On the other hand, a high level form, which depends on a plasmid-coded isoleucyl-TRNA synthetase, and which is completely resistant to mupirocin (MIC>256), is of clinical importance.[10] Although mupirocin is not of substantial therapeutic value, it plays a very important role in the control of MRSA epidemics,[2] and in prevention of infections with *S. aureus* (e.g. this study). Therefore, when mupirocin is used, the development of resistance should be monitored carefully by performing routine sensitivity tests. A second consideration is, if this form of prophylaxis in a continuous manner is the most optimal. Although resistance was not observed in this study nor in the study of Boelaert *et al.*,[4] continuous administration of any antibiotic will increase the potential for development of resistance. Holton *et al.*[12] reported an evaluation in which mupirocin was given to nasal carriers in hemodialysis for five days after which it was stopped. The efficacy of elimination of nasal carriage was lower in this study compared to our findings. The patients who had a negative nasal culture at the completion of five days of therapy (17 of 22 [77%]), had a mean time to recurrence of 3.8 weeks. During a three month follow-up period there was a significant lower rate of *S. aureus* infection in these 17 patients as compared to a control group. Further studies should be performed to evaluate which dosage schedule eliminates nasal carriage most effective, while giving a minimal opportunity for resistance to develop.

Mupirocin nasal ointment effectively eliminates nasal carriage of *S. aureus* in patients on chronic hemodialysis and thereby a significant reduction in the incidence of *S. aureus* bacteremia is achieved. Other categories of patients in which elimination of nasal carriage will likely decrease infection rates, are patients on chronic ambulatory peritoneal dialysis[15] and surgical patients.[19,20] The simplicity, low costs and efficacy of this preventive measure warrant further study.

REFERENCES

1 **Aly R, Shinefield HR, Litz C, and Maibach HI.** Role of teichoic acid in the binding of *Staphylococcus aureus* to nasal epithelial cells. *J Infect Dis* 1980;141:463-5.

2 **Barrett SP.** The value of nasal mupirocin in containing an outbreak of methicillin resistant *S. aureus* in an orthopaedic unit. *J Hosp Infect* 1990;**15**:137-42.

3 **Berman DS, Schaefler S, and Simberkoff MS.** *Staphylococcus aureus* colonization in intravenous drug abusers, dialysis patients and diabetes. *J Infect Dis* 1987;**155**:829-31.

4 **Boelaert JR, DeSmedt RA, De Baere YA, Godard CA, Matthys EG, Schurgers ML, Daneels RF, Gordts BZ, and Van Landuyt HW.** The influence of calcium mupirocin nasal ointment on the incidence of *Staphylococcus aureus* infections in hemodialysis patients. *Nephrol Dial Transplant* 1989;4:278-81.

5 **Bradley JR, Evans DB, and Calne RY.** Long-term survival in haemodialysis patients. *The Lancet* 1987;**1**:295-6.

6 **Bulanda M, Gruszka M, and Heczko B.** Effect of mupirocin on nasal carriage of *Staphylococcus aureus*. *J Hosp Infect* 1989;**14**:117-24.

7 **Chow JW, and Yu VL.** *Staphylococcus aureus* nasal carriage in hemodialysis patients. *Arch Intern Med* 1989;**149**:1258-62.

8 **Doebbeling BN, Breneman DL, Neu HC, Aly R, Yangco BG, Preston Holley H, Marsh RJ, Pfaller MA, McGowan J, Scully BE, Reagan DR, Wenzel RP and the Mupirocin Collaborative Study Group.** Elimination of *Staphylococcus aureus* Nasal Carriage in Health Care Workers: Analysis of Six Clinical trials with Calcium Mupirocin Ointment. *Clin Infect Dis* 1993;**17**:466-74.

9 **Fekety FR jr.** The epidemiology and prevention of staphylococcal infection. *Medicin* 1964;**43**:593.

10 **Gilbart J, Perry CR, Slocombe B.** High-level mupirocin resistance in *Staphylococcus aureus*: Evidence for two distinct isoleucyl-TRNA synthetases. *Antimicrob Agents Chemother* 1993;**37**:32-8.

11 **Goldblum SE, Reed WP, Ulrich JA, and Goldman RS.** Staphylococcal carriage and infections in hemodialysis patients. *Dial Transplant* 1978;**7**:1140-8.

12 **Holton DL, Nicolle LE, Diley D, and Bernstein K.** Efficacy of mupirocin nasal ointment in eradicating *Staphylococcus aureus* nasal carriage in chronic hemodialysis patients. *J Hosp Infect* 1991;**17**:133-7.

13 **Kaplowitz LG, Comstock JA, Landwehr DM, Dalton HP, and Mayhall CG.** Prospective study on microbial colonization of the nose and skin and infection of the vascular access site in hemodialysis patients. *J Clin Microbiol* 1988;**26**:1257-62.

14 **Kirmani N, Tuazon CU, Murray HW, Parrish AE, and Sheagren JN.** *Staphylococcus aureus* carriage rate of patients receiving long-term hemodialysis. *Arch Int Med* 1978;**138**:1657-9.

15 **Luzar MA, Coles GA, Faller B, Slingeneyer A, Dah G, Briat C, Wone C, Knefati Y, Kessler M, and Peluso F.** *Staphylococcus aureus* nasal carriage and infection in patients on continuous ambulatory peritoneal dialysis. *N Engl J Med* 1990;**322**:505-9.

16 **Reagan DR, Doebbeling BN, Pfaller MA, Sheetz CT, Houston AK, Hollis RJ, and Wenzel RP.** Elimination of coincident *Staphylococcus aureus* nasal and hand carriage with intranasal application of mupirocin calcium ointment. *Ann Intern Med* 1991;**114**:101-6.

17 **Tuazon CU, Perez A, Kishaba T, and Sheagren JN.** *Staphylococcus aureus* among insulin-injecting diabetic patients. An increased carriage rate. *JAMA* 1975;**231**:1272.

18 **Tuazon CU, and Sheagren JN.** Increased rate of carriage of *Staphylococcus aureus* among narcotic addicts. *J Infect Dis* 1974;**129**:725.

19 **Weinstein HJ.** The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. *N Engl J Med.* 1959;**260**:1303-8.

20 **Wenzel RP.** Preoperative antibiotic prophylaxis. *N Engl J Med.* 1992;**326**:337-9.

21 **Yu VL, Goetz A, Wagener M, Smith PB,Rihs JD, Hanchet J, and Zuravleff JJ.** *Staphylococcus aureus* nasal carriage and infection in patients on hemodialysis. *N Engl J Med* 1986;**315**:91-6.

CHAPTER THREE
EPIDEMIOLOGY

PART ONE
GLOBAL EPIDEMIOLOGY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

JAJW Kluytmans

Adapted from: **Kluytmans J.** The clonal spread of resistant organisms: methicillin-resistant *Staphylococcus aureus*. In: Proceedings of 7th Kurhaus workshop on antibiotics. Eds.: van der Meer JWM, Michel MF, Verbrugh HA. December 10, 1993;73-9.

SUMMARY

Resistance of *Staphylococcus aureus* to β-lactam antibiotics can be mediated by three mechanisms: β-lactamase production, tolerance and intrinsic resistance. Intrinsic resistance is mediated by a chromosomal gene, the Mec-a gene. This leads to the expression of an altered Penicillin Binding Protein (PBP), namely PBP-2a. This PBP-2a has a very low affinity for β-lactam antibiotics, and consequently these strains, called Methicillin-Resistant *S. aureus* (MRSA), are resistant to all β-lactam antibiotics. MRSA is now an important clinical problem in nearly all countries. In US hospitals, in 1991 MRSA-rates were 29%. In Europe the MRSA rates increase from North to South. In the Netherlands the MRSA rate is well below 5%. This low rate is maintained by surveillance and isolation of patients transferred from foreign hospitals. Two recent studies have evaluated the phylogenetics of MRSA in collections of MRSA with a global origin, gathered during the last 30 years. It can be concluded that acquisition of the Mec-a gene by *S. aureus* is an extremely rare event. Clonal spread together with horizontal transfer and recombination can explain the findings. Although the problems with MRSA are already huge, an even more worrying threat is the development of vancomycin resistance. Since the use of vancomycin is increasing and the resistance in other gram-positive organisms is already significant, the development of resistance of *S. aureus* to vancomycin is likely to occur in the near future. Further studies are needed to develop new therapeutic and preventive strategies, to deal with this totally resistant *S. aureus*.

STAPHYLOCOCCUS AUREUS: MECHANISMS OF RESISTANCE

The resistance to β-lactam antibiotics in *S. aureus* can be caused by three underlying mechanisms, namely β-lactamase production, tolerance and intrinsic resistance. The production of β-lactamase gives resistance to penicillin. The introduction of the penicillinase-stable derivatives (e.g. methicillin, cloxacillin) largely solved this problem, since these antibiotics were not affected by the staphylococcal β-lactamase. Tolerance is a mechanism first described by Tomasz *et al.*[14] in 1970. It can be observed both in vitro and in vivo. The definitions used differ somewhat. The two definitions for tolerance most often used are:

- a ratio between Minimal Bactericidal Concentration (MBC) and Minimal Inhibitory Concentration (MIC) ≥32;[13]
- a surviving fraction of ≥2% after 24 hours of incubation in the presence of high concentrations of an appropriate β-lactam antibiotic.[5]

Since tolerant strains usually do not have an increased MIC, and this test is the method most often used to detect resistance, the phenomenon is not recognised in routine sensitivity testing. The relevance of tolerance in *S. aureus* infections was recently studied by Voorn *et al.*[15] in an experimental endocarditis model in rats. The efficacies of cloxacillin, and cloxacillin combined with gentamicin, were compared for animals infected with a tolerant strain and its kill-sensitive variant. Cloxacillin was significantly less effective for treating the tolerant strain than for the kill-sensitive strain. The addition of gentamicin to cloxacillin reduced bacterial numbers in the vegetations for the tolerant strains to a level comparable to that obtained by cloxacillin alone in the kill-sensitive strain, but had no additional effect for the kill-sensitive strain. These results show that the in vitro phenomenon of tolerance is also relevant in vivo. Tolerance constitutes a strong argument for the addition of an aminoglycoside to a β-lactam antibiotic in the treatment of serious *S. aureus* infections such as endocarditis. The third mechanism of resistance is intrinsic resistance, mediated by a chromosomal gene, the Mec-a gene. This gene leads to the expression of an altered Penicillin Binding Protein (PBP), namely PBP-2a. This PBP-2a has a very low affinity for β-lactam antibiotics, and consequently these strains are resistant to all β-lactam antibiotics. Since β-lactams are the mainstay for the treatment of severe staphylococcal infections, the development of intrinsic resistance creates important problems in the therapy of these infections. These strains possessing the Mec-a gene are called Methicillin-Resistant *Staphylococcus aureus*, abbreviated to MRSA.

EPIDEMIOLOGY OF MRSA.

MRSA isolates were first reported in 1961,[7] within one year of the introduction of methicillin. Soon several epidemics were reported in a number of European countries. In the mid-1970s, MRSA was recognised as a significant clinical problem in both Europe and the US. Panlilio *et al.* reported the temporal trends of MRSA in US hospitals.[12] In 1981 the fraction of MRSA in the total number of *S. aureus* isolates was generally below 5%. In 1991 this fraction was much higher (29%). The MRSA rate correlated with the size of the hospital: in small hospitals (less than 200 beds) the MRSA rate in 1991 was 15%, in medium-sized hospitals (200 to 499 beds) the rate was 20% and in large hospitals (≥500 beds) the highest MRSA rates (38%) were found. In Asia high MRSA rates have been reported. In Japan the proportion of *S. aureus* isolates which was resistant to methicillin in 43 university hospitals was as high as 60% in 1991.[8] In China one university hospital reported a MRSA rate of nearly 80%, with a MRSA carriage rate among health care workers of 68,9%.[4] In Australia high MRSA rates (±25%) were found in Eastern regions and low rates (0,4%) in Western Australia.[3] Important

geographic differences are also found in Europe. A systematic survey showed that 12,8% of more than 7000 *S. aureus* isolates studied were methicillin-resistant. In Denmark, Sweden, Netherlands and Switzerland the MRSA-rates were lower than 2%, whereas in France, Spain and Italy rates of 30-35% were found.[16]
The low rate in the Netherlands is remarkable, especially since the rates in the neighbouring countries are much higher. In the Netherlands a huge amount of β-lactam antibiotics is used. Nevertheless, no development of resistance of *S. aureus* under therapy is observed. To put it another way, in an "MRSA-free" environment, development of resistance in the population of susceptible strains is not observed. Nearly all MRSA strains found in the Netherlands can be traced to patients transferred from foreign hospitals. There is a national guideline to control MRSA which is mainly based on surveillance and isolation of patients transferred from foreign hospitals. When MRSA is found the patient is isolated and surveillance of other patients and health care workers is performed to detect possible spreading of the strain. This policy, is called "search and destroy", and apparantly it can control MRSA-rates to a low level. The conclusion must be that development of resistance to methicillin is not or very rarely observed under the selective pressure of the use of antibiotics. Therefore, clonal spread must have been the most important mechanism causing the high rates of MRSA observed worldwide.
In contrast, development of resistance due to selection of mutants can be observed with other classes of antibiotics. This is illustrated in the following example. A patient was referred to the University Hospital Rotterdam, with a diabetic ulcer. From this ulcer two *S. aureus* strains were isolated, one being susceptible, the other resistant to gentamicin. This patient had been treated with topical gentamicin ointment. Further typing of these two strains using phage typing and randomly amplified polymorphic DNA analysis showed that the two *S. aureus* strains were identical. Apparently, resistance had developed under the selective pressure of topical gentamicin therapy, as has been described before.[17] This clearly is a different mechanism from the development of resistance to methicillin.

MRSA: PHYLOGENETICS.

From the epidemiology of MRSA it had already been concluded that inducible resistance played a very limited role. Two recent studies have evaluated the role of clonal spread of MRSA. The first study was performed by Kreiswirth *et al.*[9] They used a collection of 472 MRSA strains, which were collected worldwide during a 30-year period, the first strain being isolated in 1961. DNA hybridisation patterns were analysed, using two probes: first the Mec-a probe and second the TN-554 probe, a transposon which codes for spectinomycin resistance and is present in the majority of MRSA strains. With the Mec-a probe, six hybridisation patterns were found which, combined with the chronological sequence of isolation, fitted into one evolutionary tree. Using the TN-554 probe, 27 different patterns were identified. Each TN-554 pattern was associated with only one Mec-a pattern. The conclusions were that Mec-a may have entered *S. aureus* just once and that clonal spread together with limited horizontal transfer and recombination could explain the worldwide dissemination of MRSA.
The second study was performed by Musser *et al.*[10] They used a collection of 254 strains, also collected worldwide, but mainly in the USA, over a 30-year time span. They used multilocus enzyme electrophoresis, with selective staining for 15 different metabolic enzymes. The genetic distance between strains was calculated from the difference between the electrophoretic types (ET). Fifteen different ETs were found. At a genetic distance of 0.2 (80% homology), 6 clusters were identified. Given the differences between several ETs and the large number of different ETs found, they concluded that Mec-a had entered *S. aureus* on several occasions and that horizontal transfer and recombination explained the findings. Whatever the exact frequency, it can be concluded from the epidemiological findings,

combined with the results from both studies, that acquisition of Mec-a by *S. aureus* is an extremely rare event. Clonal spread together with horizontal transfer and recombination is the most probable mechanism, which can explain all the findings.

FUTURE CONSIDERATIONS.

MRSA is now practically resistant to all antibiotics available. The only antibiotic to which all strains are still susceptible is vancomycin (or teicoplanin). The worldwide use of vancomycin has increased dramatically over the last years. Concordantly resistance in other Gram-positive micro-organisms has increased significantly. The resistance of Enterococci in Intensive Care Units (ICUs) of US hospitals may serve as an example. In 1989, <0.5% of enterococci were resistant. In 1992, resistance had increased to nearly 8%.[6] It has been shown both in vitro and in animal studies that high-level vancomycin resistance can easily be transferred from Enterococci to *S. aureus*.[11] Therefore, it seems inevitable that this will also happen in clinical settings and vancomycin-resistant *S. aureus* (VRSA) is likelily to be a clinical problem before the turn of the century.
How to deal with VRSA? At this moment no therapeutic alternatives for VRSA are available. No new antibiotics are on the horizon. One application of an already existing drug may be the combination of amoxicillin and clavulanic acid. The theory behind this is that amoxicillin and penicillin have a high affinity for PBP-2A. Clavulanic acid inhibits the β-lactamase and therefore the combination would be an effective therapy. This is confirmed by in-vitro and in-vivo findings. In an animal model of MRSA endocarditis, ampicillin/sulbactam was an effective therapy.[2] In another study it was even more effective than vancomycin.[1] However, until now no clinical studies have been performed. Do we have to wait for the first VRSA, or should a clinical trial with less severe MRSA infections be performed now?
Another important question is, how to optimalise preventive strategies? Since VRSA most likely will have an epidemiological behaviour like MRSA, surveillance and isolation policies, as now performed in the Netherlands, could be effective. However, it is necessary to develop additional preventive strategies. Carriage plays a crucial role in the spread of *S. aureus*. Some people nearly always carry *S. aureus*, most people do so intermittently, while others never carry it at all. The underlying mechanisms are not understood and might offer a tool for new preventive measures. Besides the development of new antibiotics, prevention may be the most important way to deal with *S. aureus* infections in the near future. Further studies are needed to optimalize preventive strategies.

REFERENCES

1 **Bayer AS, Cong LI.** Ampicillin/sulbactam with rifampicin or vancomycin in treatment of experimental endocarditis due to oxacillin resistant *S. aureus. Abstract 152, 33rd ICAAC,* October 17-20, 1994, New Orleans, USA.

2 **Chambers HF, Sachdeva M, Kennedy S.** Binding affinity for penicillin-binding protein 2a correlates with in vivo activity of β-lactam antibiotics against MRSA. *J Infect Dis* 1990;**162**:705-10.

3 **Cohen SH, Morita MM, Bradford M.** A seven-year experience with methicillin-resistant *Staphylococcus aureus. Am J Med* 1991;**91(3B)**:238S-44S.

4 **Dai I, Xiang Y.** Nosocomial infections of methicillin-resistant *Staphylococcus aureus* and their detection. *Chung-Hua-I-Hsueh-Tsa-Chih* 1992;**72**:465-7.

5 **Goessens WHF, Fontijne P, van Raffe M, Michel MF.** Tolerance percentage as a criterion for the detection of tolerant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 1984;**25**:575-8.

6 **Grace Emori T, Gaynes RP.** An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev* 1993;**6**:428-44.

7 **Jevons MP.** "Celbenin" resistant Staphylococci. *Br Med J* 1961;i:124-5.

8 **Kimura A, Igarashi H, Ushioda H, Okuzuki K, Kobayashi H, Otsuka T.** Epidemiological studies of *Staphylococcus aureus* isolated from national university and medical college hospitals. *Kansenshogaku Zasshi* 1992;**66**:1543-9.

9 **Kreiswirth B, Kornblum J, Arbeit RD, Eisner W, Maslow JN, McGeer A, Low DE, Novick RP.** Evidence for a clonal origin of MRSA. *Science* 1993;**259**:227-30.

10 **Musser JM, Kapur V.** Clonal analysis of MRSA strains from intercontinental sources: association of the mec gene with divergent phylogenetic lineages implies dissemination by horizontal transfer and recombination. *J Clin Microbiol* 1992;**30**:2058-63.

11 **Noble WC.** Transfer of vancomycin resistance to methicillin-resistant *Staphylococcus aureus. FEMS Microbiol Lett* 1992;**93**:195-8.

12 **Panlilio A, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, Martone WJ and the National Nosocomial Infections Surveillance System.** Methicillin-resistant *Staphylococcus aureus* in US hospitals, 1975-1991. *Inf Control Hosp Epidemiol* 1992;**13**:582-6.

13 **Sabath LD, Wheeler N, Lavardiere M, Blazevic D, Wilkinson BJ.** A new type of penicillin resistance of *Staphylococcus aureus. Lancet* 1977;i:443-7.

14 **Tomasz A, Albino A, Zanati E.** Multiple antibiotic resistance in a bacterium with suppressed autolytic system. *Nature* 1970;**227**:138-40.

15 **Voorn GP, Thompson J, Goessens WHF, Schmal-Bauer WC, Broeders PHM, Michel MF.** Role of tolerance in cloxacillin treatment of experimental *Staphylococcus aureus* endocarditis. *J Infect Dis* 1991;**163**:640-3.

16 **Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I.** Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect Dis* 1994;**13**:211-8.

17 **Wyatt TD, Ferguson WP, Wilson TS, h/lcCormick E.** Gentamicin resistant *Staphylococcus aureus* associated with the use of topical gentamicin. *J Antimicrob Chemother* 1977;**3**:213-7.

CHAPTER THREE
EPIDEMIOLOGY

PART TWO
EPIDEMIOLOGY AND RISKS ASSOCIATED WITH NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*

Jan Kluytmans, Alex van Belkum, Henri Verbrugh

Adapted from: *Clin Microbiol Rev* In press.

INTRODUCTION

Staphylococcus aureus has long been recognized as an important pathogen in human disease. Staphylococcal infections occur regularly in hospitalized patients and have severe consequences despite adequate antibiotic therapy.[90,218] Due to an increasing number of infections caused by methicillin resistant *S. aureus* (MRSA) strains, which are now most often multi-resistant, therapy has become problematic.[205] Therefore, prevention of staphylococcal infections is now more important. Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection. This review, discusses the literature on the epidemiology of nasal carriage and the risks associated with it. In addition to presenting what is known at this time it also focuses on studies that need to be performed in order to develop optimal preventive strategies.

CARRIAGE OF *S. AUREUS*

Epidemiology. The ecological niche of *S. aureus* strains are the anterior nares. Elegant studies have shown that the nares are the most reliable area to isolate this organism.[210] Moreover, when the nares were treated topically to eliminate nasal carriage, in most cases the organism also disappeared from other areas of the body.[145,158] Over time, three patterns of carriage can be distinguished. Approximately 20% of individuals almost always carry one strain and are called persistent carriers. A large proportion of the population (±60%) harbors *S. aureus* intermittently and change strains with varying frequency. Such persons are called intermittent carriers. Finally, a minority of people (±20%) almost never carry *S. aureus* and are called non-carriers.[210] Persistent carriage is more common in children than in adults and many people change their carrier-type between the age of 10 and 20 years.[11] The reasons for these differences in colonization patterns are as yet unknown. Persistent carriage seems to have a protective effect on the acquisition of other strains, at least during hospitalization.[138] This barrier to colonization is reduced when carriers are treated with antibiotics.[138] These findings suggest that the acquisition and transmission of antibiotic-resistant *S. aureus* in the hospital mainly concerns intermittent carriers and persistent carriers treated with antibiotics. The prevalence and incidence of *S. aureus* nasal carriage varies according to the population studied. The results of studies on nasal carriage as determined in cross-sectional surveys are shown in Table 1. In the general population a mean carriage rate of 37.2% was found. However, the range of carriage rates reported is large. This may partly be due to differences in the quality of the sampling and of the culturing techniques used in these studies. Also, the studies were reported between 1934 and 1994, and changes in *S. aureus* nasal carriage may have occurred over the years. The older studies tend to find higher carriage rates. The rates in the general population are comparable to those found in health-care workers and in patients on admission and during hospitalization (see Table 1). Some studies however, reported increased carriage rates when patients were hospitalized (Table 2). Subgroups of patients with significantly increased carriage rates include those with insulin dependent diabetes mellitus, on hemodialysis, or continuous ambulatory peritoneal dialysis (CAPD), intravenous drug addicts, patients with *S. aureus* skin infections, and those with HIV/AIDS (see Table 1). In the first four groups, the common factor seems to be repeated or long-term puncture of the skin by needles and/or intravascular catheters. To confirm this association, higher carriage rates were also found among relatively healthy patients receiving repeated allergy injections.[69] In patients with *S. aureus* skin infections it is predictable that high nasal carriage rates are found since nasal cultures were taken at the time that *S. aureus* infection was present. However in HIV-positive patients the reason for the higher carriage rates is unclear. The studies on HIV cited in Table 1 have excluded HIV-positive intravenous drug

addicts since their carriage rate would be expected to be high even in the absence of HIV infection. Furthermore, the stage of HIV disease did not seem to influence the carriage rate. To control for hospitalization as a risk factor, Weinke *et al.*[207] selected patients with other types of chronic disease as controls, but nevertheless, the carriage rate in HIV-positive patients was higher. They proposed that immunological defects were the basis for the higher carriage rate in HIV patients. However, the exact nature of these defects remains to be elucidated. In addition to the groups mentioned above there have been some anecdotal reports on other groups with high *S. aureus* carriage rates. Decker *et al.*[40] found a carriage rate of 65.6% in river rafting guides. In this group the *S. aureus* infection rates were also high. Maceration of the skin caused by prolonged contact with water, together with repeated small skin injuries, were the proposed reasons. Finally, Gittelman *et al.*[60] found high carriage rates in patients with rhino-sinusitis.

Table 1: *Rates of S. aureus nasal carriage in various populations.*[†]

Population	N	carriage rate		references
		mean	range	
General	13,873	37.2%	19.0-55.1	27, 38, 55, 62, 83, 99, 118, 123-125, 135, 137, 162, 178, 186, 213
Health care workers	2,568	26.6%	16.8-56.1	37, 46, 98, 100, 113, 144, 162
Patients on admission*	21,842	35.7%	10.2-85.0	37, 70, 100, 138, 160, 163, 184, 212
Patients hospitalized	3,879	29.8%	14.3-52.5	14, 98, 100, 102, 108, 144, 153
Diabetes mellitus				
insulin dependent	454	56.4%	24.1-76.4	15, 107, 122, 178, 194
non-insulin dependent	434	29.0%	11.1-35.0	27, 107, 178, 194
controls	1,035	32.2%	9.1-44.3	27, 107, 178
Dialysis				
Hemodialysis	454	51.5%	30.1-84.4	15, 61, 85, 88, 131, 159, 193, 217
CAPD	605	43.3%	16.8-51.4	39, 110, 142, 150, 171, 172
Chronic renal failure only	38	21.1%	14.3-33.3	61, 88, 193
Drug addicts (DA)				
Intravenous DA	288	55.2%	33.8-61.4	15, 195
non-intravenous DA	603	25.9%	9.1-49.0	15, 195
***S. aureus* skin lesions**	1,439	65.9%	42.0-100	72, 101, 134, 182
HIV/AIDS				
HIV positive	664	35.5%	26.9-54.7	10, 13, 59, 157, 207
controls (HIV negative)	508	20.9%	17.2-30.8	10, 13, 59, 157, 207

[†] Only studies in which prevalence rates of nasal carriage of *S. aureus* were determined are included.
* For admission rates of surgical patients see also Table 3.

Molecular basis of the carrier-state. Although *S. aureus* can be cultured from multiple sites of the skin and mucosal surfaces of carriers, the primary reservoir of staphylococci is thought to be the *vestibulum nasi* or anterior nares, the nostrils of the nose. Inside, this part of the nose is lined by a fully keratinized epidermis that presents hairs, sebaceous glands and sweat glands. The vestibule is limited above and behind by a ridge, the *limen nasi*, over which the skin becomes continous with the nasal mucous membrane. Apparently, the staphylococcal cells flourish here in the relative absence of human defenses and/or they are capable to withstand the local antibacterial defenses. In order to adhere, bacterial cells need to establish firm interactions with the various types of human cell surfaces, thus preventing their rapid elimination by physicochemical mechanisms. To establish successful colonization it is thought that surface components of the staphylococcal cell interact with counter components on the membranes of the eukaryotic host cells. Bacterial adherence may be non-

specifically mediated via physicochemical forces including hydrophobic interactions.[35] Alternatively, adherence may more specifically be accomplished through binding of certain bacterial cell surface moieties (adhesins) to defined structural receptors in the membrane of the host cell. A wide array of staphylococcal and host cell determinants have been studied at the molecular level in a similarly diverse array of test systems. Table 2 shows factors that have been found to be associated with carriage of *S. aureus*. There appear to be differences in adherence between *S. aureus* strains and between nasal epithelial cells from different individuals. *S. aureus* has a greater affinity for nasal epithelial cells obtained from carriers than from noncarriers.[5] In addition, *S. aureus* adheres better to nasal epithelial cells from patients with eczema than to cells from patient without eczema.[3] Also nasal carriage may be associated with certain HLA antigens such as DR3, but not with others.[87]

Table 2: *Factors which may influence the rate of S. aureus nasal carriage.*

Factor	references
Adherence to epithelia which is mediated/influenced by:	
Lipo-teichoic acid in *S. aureus* cell wall	2, 30
Surface associated proteins in *S. aureus* cell wall	56
Carriers versus non-carriers	5
Viral infections of the upper respiratory tract	51
Nasal abnormalities	83
HLA type	87
Ecology of nasal flora	104, 119
Race	124, 135, 162
Age	11, 138
Genetic	77
Immunological factors	47
Repeated needle injections	88, 89, 195
Hormonal status in women	213
Hospitalization	62, 136, 144, 176, 212

This section will focus on the interaction of *S. aureus* with epithelia, especially from the airways. Also included are studies of their interaction with other cells including the endothelial and mesothelial cells that line the vascular bed and major body cavities, respectively. The adherence of microorganisms to extracellular matrices of the body was recently reviewed elsewhere.[156]

Adherence to airway epithelium. Staphylococci have been shown to adhere well to cells scraped from the anterior nares of healthy volunteers,[6,9,169,177,205] patients with dermal pathologies[7] and geriatric patients.[161] The molecular basis of staphylococcal adherence to this site has only partially been elucidated. Host factors are important since early studies have shown that recolonization with strains that had been present before is virtually impossible. This rejection after reinoculation is indicative for a local immune response.[47] The involvement of age and genetic background was shown in these and other studies.[8,87] Epithelial antibiotic substances have recently been discovered and they may play a role in the prevention of the *S. aureus* carrier state.[168]

Eukaryotic surface glycoproteins and proteoglycans, present on the mucous membranes, contribute to the adhesion of bacteria. There is considerable heterogeneity among nasal cells in participating in the adherence of bacteria. Mucin-coated cells seem to adhere staphylococci much better than cells without such carbohydrate coat.[177] Staphylococci will

bind to bovine mucin and to mucus in a ferret model of adherence,[166,191] but they do not seem to adhere to the ciliated cells of the airway epithelium. Other substances found in the respiratory tract including secretory IgA,[16] glycolipids[97] and surfactant protein A[115] may also constitute receptor sites for staphylococcal adherence. Interestingly, *S. aureus* was recently found to adhere better to a bronchial cystic fibrosis cell line (genotype ΔF508/w1282X) than to matched rescued control cells. Increased binding was to the tetrasaccharide [Galβ1-3GalNacβ1-4Gaβ1-4Glc] of the asialoganglioside 1 (aGM1). More aGM1 is produced and excreted apically by cystic fibrosis epithelia because these cells have impaired sialyltransferase activity.[62] The importance of carbohydrates in receptor molecules on the surface of epithelial cells is further evidenced by the loss of staphylococcal adherence to periodate-treated mucins.[177] However, hydrophobic interactions and surface charge provide forces that are likely involved as well in mediating staphylococcal binding to epithelia.[30,169,177] There is significant interindividual variation with higher rates of staphylococcal binding being observed for carriers than for non-carriers of *S. aureus*,[6] for older babies than for neonates in the first week of life,[9] for Influenza A virus-infected volunteers compared to control uninfected cells[51] and for moderately ill geriatric patients compared to elderly patients that are seriously ill.[161] The dynamic processes underlying these variations in staphylococcal adherence are largely unknown. In an in vitro system using canine kidney cells Sanford *et al.*[165] found Influenza A virus to induce more and new plasmamembrane receptors for *S. aureus* binding; these receptors were distinct from the hemagglutinins that are also induced by the virus.

There is no consensus on the surface components of *S. aureus* that mediate binding to epithelial membranes. Cell wall teichoic acid, lipoteichoic acid, fibronectin binding proteins, heat-labile and heat-extractable proteins, and even type 5 and type 8 capsular polysaccharides have been proposed as major ligands.[9,30,165,181] Purified capsular polysaccharides bound well to a human epithelial carcinoma cell line in vitro and induced these cells to produce cytokines.[181] However, there is no direct evidence of capsule-mediated adherence of staphylococci to epithelial cells. In studies using nasal epithelial cells free teichoic acid blocked binding of staphylococci to fully keratinized cells whereas fibronectin partially blocked adherence to keratinized cells but not to spinous or low granular epithelial cells,[9] probably by masking teichoic acid binding sites. Staphylococcal adherence is better to the fully matured, i.e. keratinized, cells than to granular or spinous cells present deeper in the epidermis.[9] However, pretreatment of the bacteria with proteolytic enzymes, with heat (> 100 °C) or with the protein synthesis inhibiting antibiotic clindamycin will reduce their adherence to epithelia and to mucin indicating that surface proteins may be involved as well.[6,30,174,177] *S. aureus* protein A is probably not involved since isogenic *spa*-mutants adhered as well as the parent strain to Hep-2, Vero and mesothelial cell monolayers.[154] Mutation to *agr*-, however, increased staphylococccal adherence twofold indicating that a cellular product is involved that is produced during the exponential phase of growth; the gene for this product is in the vicinity of the *mec* gene.[154] In contrast to the adhesins for cellular surfaces, protein A and other surface proteins that are adhesins for extracellular matrixproteins have been characterized in detail; these adhesins recognize, among others, fibronectin, fibrinogen and collagen, and have been described as 'microbial surface components recognizing adhesive matrix molecules' (MSCRAMMs)(see reference 146).

Adherence to bovine mammary gland epithelium. Since *S. aureus* is one of the major pathogens causing mastitis in cattle its adherence to epithelia from the bovine mammary has been studied in some detail. *S. aureus* adheres well, better than most other bacterial species, to these epithelial cells. Especially to cells higher up in the gland. Again, not all cells participate in the binding of staphylococci; staphylococci only attach to the non-villiated, domeshaped, hexagonal cells that seem capable of secretion.[58] The adherence of bacteria to the keratinized cells of the teat canal varies with the origin of the cells,[187] and is reduced by

pretreatment of the bacteria with proteolytic enzymes. Recently a cell wall protein of 145 kDa was isolated from a strain of *S. aureus* that could bind to mammary epithelial cells.[105] Interestingly, the adhesion of the bacteria becomes enhanced when bacteria are grown in milk-whey instead of in broth. In this circumstance adherence is trypsin-resistant but periodate-sensitive indicating that the putative adhesin(s) is of a largely carbohydrate nature.[112] Adhesion of staphylococci to fat globules in milk has also been demonstrated, and it is proposed that such binding promotes dissemination of the bacteria in the mammary gland. Although antibody against whole bacteria can block staphylococcal adherence to epithelial cell lines derived from high ductular tissues, the surface epitopes involved in binding have not been further delineated.[141] Thus, the relative contributions of non-specific physicochemical interactions and specific interactions between staphylococcal cell wall-associated moieties and host receptors remain unknown.

Adherence to endothelium and mesothelium. Staphylococci regularly invade the bloodstream and the large body cavities where they encounter endothelial and mesothelial cells, respectively. Again, *S. aureus* avidly binds to these cellular surfaces and may even enter the cells.[69,140] Early studies by Gould *et al.* showed extensive staphylococcal adherence to canine and human cadaveric heartvalves.[63] Binding to the surface of endothelial cells is probably mediated to a large extent by fibronectin since this endothelium-derived molecule is ubiquitously present in the vascular space and *S. aureus* has adhesins for this protein.[201,203] Fibronectin seems to bind an uronic-amine exopolysaccharide at some distance from the electron-dense cellwall. Fibronectin binding to teichoic acid has been reported.[9] Interestingly, data suggest that efficient binding of *S. aureus* to endothelium is an active process which likely requires the synthesis of proteins by the endothelial cells.[190] Thus, staphylococcal adherence is reduced at 4°C and if endothelial cell have been preincubated with dactinomycin; adherence increases when cells are prestimulated by interleukin-1. Adherence can also be modulated by growth factors.[20]

The role of fibronectin in mediating *S. aureus* adherence to mesothelial cells is in doubt.[69,154] It has been shown that anti-fibronectin antibody reduces adherence of *S. aureus* to fibronectin to a greater extent than to mesothelial cells suggesting an additional receptor site on mesothelial cells. Free lipoteichoic acid consistently interfered with staphylococcal binding to mesothelial cell monolayers making this cell wall component a likely adhesin-candidate.[69]

In summary one can conclude that the molecular determinants of *S. aureus* adherence to human epithelia remain to be fully described. As long as such insight into *S. aureus* adherence is lacking, a valid explanation for the carrier state in some individuals and not in others, cannot be given.

Carriage of MRSA. Risk factors for acquisition of MRSA include the administration of multiple antibiotics.[26,36,147] The nasal bacterial flora is modified when systemic antibiotics are given.[4] Interestingly, older data indicate that increased environmental contamination with penicillin was an important risk factor for colonization of the nares of hospitalized patients with penicillin-resistant staphylococci or for the transmission of penicillin-resistant *S. aureus* to other patients.[133] It has also been shown that administration of tetracycline to patients colonized with a tetracycline-resistant strain of *S. aureus* induced dispersal of the organism in the environment[18] thus contributing to their further spread. MRSA are usually resistant to several groups of broad-spectrum antibiotics which are used on a large scale in the hospital. This mechanism of increased spreading under antibiotic pressure may have contributed to the worldwide increase of the prevalence of MRSA in hospitals.[205]

Molecular typing of *S. aureus* strains from carriers. An important topic in nasal colonisation studies concerns the population characteristics and dynamics of the resident *S. aureus* strain(s). Major questions are whether or not a persistently colonised individual is

always inhabiting the same strain, whether strain exchange or replacement can be observed, and if persistent strains share certain genetic characteristics which set them apart from the strains that display intermittent colonisation only. For answering these questions, medical microbiologists have a broad spectrum of technical instruments at their disposal. Varying from techniques that monitor phenotypic characteristics to those that involve genetic procedures which highlight DNA polymorphisms (for a review, see reference 120). Testing of sensitivity to restriction-sensitive bacteriophages is, among the phenotypic procedures, considered to be the golden standard. Unfortunately, most laboratories are not equipped to handle phage typing, and this technique can only be employed succesfully if performed in an experienced reference laboratory.[120] Even in this setting a number of strains can not be evaluated since they are untypable. DNA-typing procedures are easier to perform and most are able to type all strains. Although more easy to handle than phage typing, they are not yet available in most routine microbiology laboratories.[192] Genotypic procedures can lead the way to elucidating the molecular genetics of colonising *S. aureus* populations. Especially DNA-typing procedures are being applied with increasing frequency and have been proven quite valuable for assessing clonality among given strains of many bacterial species including (methicillin-resistant) *S. aureus*.[133] For staphylococci in general, comparative molecular typing has been the subject of a number of recent studies.[34,189,197] From these studies it was deduced that DNA analysis, either by PCR mediated techniques or pulsed-field gel electrophoretic separation (PFGE) of DNA macrorestriction fragments, can be applied successfully once the techniques have reached a sufficient level of reproducibility. This is generally coupled to an excellent degree of resolution among non-related strains, whereas epidemiologically clustered isolates are identified as being identical. Using these relatively novel techniques in studies of nasal populations of *S. aureus* may provide interesting new insights. At present this type of studies is still limited in number (see below). Although most epidemiological studies on *S. aureus* focus on monitoring the spread of particular strains,[92,173] sometimes strains isolated from the nasal cavities of multiple persons have been analyzed. A recent Japanese study revealed that within individual carriers the same strain could be identified upon every positive culture.[60] Although this is in contradiction with old data obtained by phage typing of staphylococcal isolates,[71] the authors suggest that recolonisation of the anterior nares rarely occurs. It is also suggested that bacterial interference may lead to non-carriership: when an ecological niche is already occupied, for instance by coagulase negative staphylococci, *S. aureus* does not have the means to replace the resident bacterial population. A recent study from our laboratory[193] revealed that among Danish volunteers, who were longitudinally examined for nasal carriership of *S. aureus*, several "carrier states" could be distinguished. Persons could be classified as either persistent (>8 positive cultures upon 10 examinations) or intermittent (60-80% positive cultures) carrier; occasional carriers were positive on 10-40% of the occasions only. All strains were analyzed phenotypically by phagetyping and by measuring the amount of surface-exposed protein A. Genetic typing was done with random amplification of polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) analysis of the coagulase and protein A genes.[57,170] Based on all results no common genetic or phenetic characteristics segregating persistent from intermittent or occasional colonisers could be identified. Both protein A and coagulase gene polymorphisms did not correlate with the type of carrier state. Apparently, polymorphisms in these genes do not seem to play a significant role in the propensity of *S. aureus* to colonise human nasal epithelium.

The number of studies on the molecular epidemiology and population dynamics of nasal populations of *S. aureus* are still limited in number. Additional analyses are required, the data of which may open novel pathways for elimination of resident strains.

Application of molecular typing techniques in prevention of nosocomial S. aureus infections. Another application of typing techniques is to elucidate the epidemiology of S. aureus infections in the hospital environment. Historically, *S. aureus* has been an example of cross-infection.[211] Carriers among health care workers or patients have been identified frequently as the source of outbreaks. However, other data show that many *S. aureus* infections have their origin in the patients' endogenous flora (see other parts of this review). How to deal with *S. aureus* infections in the hospital setting? The basis of infection control is surveillance. This means that the rates of nosocomial infections should be monitored on a permanent or semi-permanent basis.[96] In case of increased rates, either epidemic or endemic, the isolates cultured from infections should be typed. In order to be able to do this, the microbiology laboratory should store all relevant clinical isolates routinely, for some time. When typing results show that there is a high level of clonal relatedness among the strains, the investigation should be aimed to identify a common source. However, if there is no relatedness among the strains, it is unlikely that there is a common source and therefore, the investigation should be directed into another way, for instance looking for breaks in hygienic procedures or surgical techniques. For these kind of investigations molecular typing methods have proven to be fashionable and reliable. Also it is possible to type nearly all kinds of pathogenic micro-organisms using only one procedure.[199,200] The application of typing procedures has enabled to target epidemiological investigations, and is an important instrument in the prevention of nosocomial infections.

CARRIAGE OF *S. AUREUS* AS A RISK FACTOR FOR INFECTION.

Carriage of *S. aureus* has been identified as a risk factor for the development of infections in various settings (see Table 3 and 4). This has been studied extensively in surgical patients, in patients on hemodialysis and, to a lesser extent, in patients on CAPD. Also, anecdotal reports have been published for intravascular device-related bacteremia, *S. aureus* bacteremia in HIV-positive patients and about a higher relapse rate in patients with Wegener granulomatosis. Carriage of MRSA constitutes a special problem with regard to prevention and treatment of infection. Elimination of nasal carriage would theoretically reduce the infection rates in populations in which it has been identified as a risk factor. The literature available on these subjects will be reviewed in the following sections.

Surgery. Probably, one of the first observations pointing to the endogenous source of bacterial wound infection was made in 1915 by Sir Almroth E. Wright.[216] In a report on wound infection during world war I, he made the following statement: "During this war every wound is heavily infected. The chain of cause and consequence seems to be as follows: The clothes and skin of the soldier on war service become contaminated with all manner of filth containing pathogenic organisms and spores; the projectile takes these in with it, and it implants them far in-in point of fact, far beyond the reach of any prophylactic applications of antiseptics". This description is not entirely of an endogenous infection because it implies that most of the infecting organisms were contaminating the skin and clothes before they were inserted and, thus, were not part of the soldiers' endogenous flora. Nevertheless the description of the route of infection is clearly endogenous. In surgical procedures this transmission-route is prevented largely by disinfection of the surgical site before incision and covering the patient with sterile drapes. Despite these preventive measures a clear correlation exists between carriage of *S. aureus* and the development of *S. aureus* surgical wound infections. This relation was established first in the late 1950's. The major reason for the studies in those years was a pandemic of serious staphylococcal infections both in the community and in hospitals,[128] which involved strains that were resistant to many of the antibiotics available at that time. Resistance was considered to enhance the spread and virulence of *S. aureus* in the

hospital.[43,211] A great number of studies were performed to gain insight into the epidemiology of *S. aureus* in both the community and in the hospital in order to develop preventive strategies. For those purposes it was important that phage typing was available, enabling comparison of *S. aureus* isolates. Cross-infection was the primary focus of attention and much was learned about the relative importance of patients, health-care workers and the environment (both fomites and air) in the transmission of *S. aureus* (For a comprehensive review see reference 211). At some point, the observation was made that some patients developed infections with *S. aureus* strains that had been isolated previously at other sites from the same patient and not from other potential sources in the environment. In 1959, three independent reports were published which had investigated primarily the relation between nasal carriage of *S. aureus* and the development of surgical wound infections.[33,208,212] In the next decennium a number of studies followed, most of which showed a significantly increased risk for development of a wound infection by nasal carriers as shown in Table 3. The causal relationship was strengthened by showing a correlation between the colonization density of *S. aureus* at the carriage site and the risk for the development of infection as demonstrated by Calia *et al.*,[29] White *et al.*,[209] Bruun,[28] and Kluytmans *et al.*[94] Typing of *S. aureus* showed that a variable proportion (30 to 100%) of infections was due to endogenous strains (Table 3). The variations between the risk-ratios and proportions of infections due to endogenous strains in various studies may be due to differences between the patient populations, the types of surgery performed, and the perioperative care such as preoperative length of hospitalization, antisepsis, asepsis and the perioperative use of systemic antibiotic prophylaxis. These factors can influence the overall infection rate, the risk of cross-infection, and probably also the degree of endogenous infection. Also the sensitivity of the culture technique employed has to be taken into account. As mentioned before the risk of infection correlates with the number of staphylococci in the nose. Therefore, studies which use a very sensitive culture technique will identify more carriers which are not at increased risk, which will result in a lower relative risk. In spite of these differences, it is clear that there is a certain amount of endogenous infection with *S. aureus* in nearly every surgical setting. Considering the results of the most recent studies, the proportion of endogenous *S. aureus* infection as opposed to cross-infection may even be higher today than formerly. If the results from the 14 studies performed untill 1970, shown in Table 3, are combined there were 467 *S. aureus* infections in 5030 carriers and 348 in 6844 non-carriers. This results in a relative risk of 1.8 with a 95% confidence interval from 1,6 to 2.1 for carriers. In the four studies performed in the 1990s, 50 infections were observed in 628 carriers and 33 in 2962 non-carriers, giving a relative risk of 7.1 with a 95% confidence interval from 4.6 to 11.0. It is not unreasonable to state that the risk of cross-infection in the setting of modern hospitals is lower than in the earlier investigations. Important improvements have been made in the design of the operating theater, the surgical procedures, the administration of perioperative antibiotic prophylaxis, and aseptic perioperative care. Therefore, the current importance of nasal carriage of *S. aureus* as a risk factor in various populations of surgical patients, deserves further evaluation since it could have important implications for prevention.

Hemodialysis. Infection is a major cause of morbidity and the second most common cause of mortality in hemodialysis patients[168] *S. aureus* is the most frequently isolated pathogen, causing infection at the vascular access site which is often associated with bacteremia. In Table 4, four studies are shown which evaluated the importance of nasal carriage for the development of *S. aureus* infections in patients on hemodialysis. The infection rate was higher in carriers in all studies, with relative risks varying from 1.8 to 4.7. In another study *S. aureus* isolates in carriers and those isolated from the site of *S. aureus* infection were typed (phage- and plasmid-typing) and compared.[49] From 12 infectious episodes, 11 *S. aureus* isolates were identical to the one previously isolated from the patients nares. It can be

Table 3: *Nasal carriage of S. aureus as a risk factor in surgical patients.*

Reference				Infections/patients				
no	year	no patients	*S. aureus* carriage-rate (%)	carriers	non-carriers	Relative risk	95% confidence interval	Identical types in carriers
33	1959	348	30	15/104	5/244	7,0	2,6 - 18,9	100%
208	1959	125	34	16/43	9/82	3,4	1,6 - 7,0	91,7%
212	1959	1319	52	47/687	13/632	3,3	1,8 - 6,1	59,6%
155	1960	3056	27	73/821	158/2235	1,3	1,0 - 1,6	42,9%
73	1961	413	46	15/190	4/223	4,4	1,5 - 13,0	nd
116	1961	187	40	12/74	11/113	1,7	0,8 - 3,6	nd
12	1963	520	85	24/442	6/78	0,7	0,3 - 1,7	58%
		2480	55	30/1371	25/1119	1,0	0,6 - 1,7	30%
74	1963	100	68	6/68	2/32	1,4	0,3 - 6,6	50%
79	1963	330	17	6/57	12/273	2,4	0,9 - 6,1	33,3%
86	1963	430	42	57/181	15/249	5,2	3,1 - 18,9	94,7%
209	1963	451	23	20/106	28/345	2,3	1,4 - 4,0	nd
			9 light	4/42	28/345	1,2	0,4 - 3,2	
			6 moderate	5/26	28/345	2,4	1,0 - 5,6	
			8 heavy	11/38	28/345	3,6	1,9 - 6,6	
106	1967	146	46	25/67	16/79	1,8	1,1 - 3,2	nd
29	1969	269	36	16/96	16/173	1,8	0,9 - 3,4	100%
			11 light	2/29	16/173	0,7	0,2 - 3,1	
			12 moderate	3/31	16/173	1,0	0,3 - 3,4	
			13 heavy	11/36	16/173	3,3	1,7 - 6,5	
28	1970	2260	48	104/1093	28/1167	4,0	2,6 - 6,0	nd
			15 heavy	65/336	28/1167	8,1	5,3 - 12,3	126
	1993	306	15	8/47	4/259	9,4	2,9 - 30,2	91%
90	1995	1980	13	21/264	19/1716	7,2	3,9 - 13,2	100%
164	1996	1049	24	15/248	8/801	6.1	2.6 - 14.1	87%
94	1996	255	27	6/69	2/186	8.1	1.7 - 39.1	nd
			9 light	0/23	2/186	na		
			18 heavy	6/46	2/186	12.1	2.5 - 65.4	

Table 4: *Nasal carriage as a risk factor in other categories of patients.*

reference no	reference year	no patients	carriage rate (%)	site of infection	carriers+	non-carriers+	RR	95% CI	remarks
CAPD									
172	1982	30	33	exit site	8/10	4/20	4.0	1.6 - 10.1	
				peritonitis	7/10	1/20	14.0	2.0 - 98.7	
39	1989	87	23	exit site	14/20	7/67	6.7	3.1 - 14.3	
171	1989	43	65	peritonitis	16/28	0/15	n.a.		
110	1990	140	45	exit site	22/63	2/77	13.4	3.3 - 55.0	
				peritonitis	11/63	0/77	n.a.		
				tunnel	5/63	2/77	3.1	0.6 - 15.2	
150	1993	138	51	exit site	81/71	31/67	2.1	1.7 - 2.8	
				peritonitis	38/71	9/67	4.0	2.1 - 7.6	
				tunnel	23/71	12/67	1.8	1.0 - 3.3	
111	1993	167	17	all	24/28	18/139	6.6	4.2 - 10.5	MRSA carriage and infections only
Hemodialysis									
159	1975	32	84	access	8/27	1/5	1.8	0.3 - 11.7	
61	1978	40	35	all	10/14	10/26	1.9	1.1 - 3.3	Skin carriage was evaluated
217	1986	86	70	all	12/26	3/26	4.0	1.3 - 12,5	34 carriers are not included since they were treated for elimination of carriage
85	1988	70	51	access	5/36	1/34	4.7	0.6 - 38.4	
HIV/AIDS									
207	1992	136	53	bacteremia	8/72	0/64	n.a.		
Intravascular device associated bacteremia									
180	1990	107	14	bacteremia	7/15	6/86	6.3	2.6 - 17.2	
156	1996	488	30	bacteremia	32/147	6/341	12.4	5.3 - 29.0	
MRSA in long-term care									
129	1991	197	16	all	8/32	6/132	5.5	2.0 - 14.7	
MRSA in intensive care									
121	1994	484	4	all	5/19	6/465	20.4	6.8 - 61.0	
156	1996	488	13	bacteremia	24/63	8/84	4.0	1.9 - 8.3	MRSA-carriers versus MSSA-carriers
					24/63	6/341	21.7	9.2 - 50.8	MRSA-carriers versus non-carriers
Relapse rate in Wegener granulomatosis									
183	1994	57	63	relapse rate	21/36	2/21	6.1	1.6 - 23.6	

+ Episodes of *S. aureus* infection/number of patients

concluded that patients on hemodialysis have an increased *S. aureus*. carriage rate and that most *S. aureus* infections in this setting are of endogenous origin.

CAPD. In patients treated with CAPD, *S. aureus* infections constitute frequent and serious problems, being the leading cause of exit site- and tunnel-infection, often leading to catheter loss.[109,151,220] The first study reporting an association between carriage of *S. aureus* and an increased infection rate in CAPD was performed by Sewell *et al.*, in 1982.[172] Several others followed with the results shown in Table 4. The observed relative risks for carriage are even higher than in hemodialysis patients (range: 1.8 to 14.0). As shown in Table 4 several studies looked at the risk for infection at various sites (exit site, peritoneum, and tunnel track). All sites were at increased risk in carriers. In addition, some studies used typing methods to compare *S. aureus* isolates from carriage sites with isolates from infections. Luzar *et al.*[110] found identical isolates in 85% of the patients with infections. Pignatari *et al.*[149,150] showed that in 6 patients with 8 episodes of peritonitis caused by *S. aureus*, all isolates from the site of infection were identical to strains isolated previously from carriage sites. The same observation was made by Sesso *et al.*[171] in 8 patients with *S. aureus* peritonitis. These results show that patients on CAPD are like those on hemodialysis, in that the carriage rate is high and that *S. aureus* carriage is an important risk factor for the development of *S. aureus* infections.

HIV, AIDS-related complex (ARC) and AIDS. In patients with ARC and AIDS, increased rates of *S. aureus* bacteremia, deep soft tissue infections and recurrent *S. aureus* infection have been observed.[84,179,214] In one of these studies, observations were made in a group of patients without other known risk factors for *S. aureus* infection (e.g. no intravenous drug abuse). Theoretically the higher infection rates may be due, in part, to impaired B-cell immune function. However, Ganesh *et al.*[59] found a higher carriage rate of *S. aureus* in asymptomatic HIV-positive homosexuals compared with HIV-negative homosexuals (carriage rates: 49 and 27%, respectively, $P<0.05$). Weinke *et al.*[207] studied the carriage rates in 136 HIV-positive patients with various stages of progression of disease (23.5% asymptomatic, 26.5% ARC, and 50% AIDS). The carriage rate was somewhat lower in homosexuals (40.2%) than in intravenous drug addicts (52.9%), giving an overall rate of 44.1%. The rate was not influenced by the CD4 cell count nor by granulocytopenia. Nevertheless, a higher rate was observed in patients with ARC or AIDS compared with HIV-positive, asymptomatic patients. In other hospitalized patients with various chronic diseases, a carriage rate of 30.8% ($P>0.05$) was found and in hospital staff the rate was 23.4% ($P<0.05$). Therefore, the increased *S. aureus* infection rates in HIV-positive patients could in part be related to the high carriage rates of *S. aureus* in these patients. In the same report, it was found that *S. aureus* septicemia occurred only in carriers (Table 4) which was statistically highly significant. Therefore, in HIV-positive patients carriage rates are also increased and this is a risk factor for subsequent infection with *S. aureus*. It should be noted that the carriage rates in HIV-positive patients may even be higher than those actually observed if there was not such a widespread use of antibiotic prophylaxis (e.g., cotrimoxazole for *Pneumocystis carinii*) and antibiotic therapy in this particular group of patients. The growing number of HIV patients warrants more studies to establish the importance of nasal carriage for development of infection in this group of patients. Furthermore, the underlying mechanism of increased carriage in this group of patients remains to be elucidated.

Intravenous device associated bacteremia. After coagulase-negative staphylococci, *S. aureus* is the second most prevalent organism causing intravenous device-associated bacteremia.[117,167] However, no study has been performed with the primary aim of establishing the role of *S. aureus* nasal carriage in this important setting. Lipsky *et al.*[103] have studied the relationship between nasal carriage and intravenous therapy-related phlebitis. They did not

find a correlation, but commented that most of their cases of phlebitis were not infectious but physicochemical phenomena. In Table 4 the results from a study by Snydman *et al.*[180] are shown. This study evaluated the incidence and risk factors for the development of nosocomial bacteremia in patients treated intravenously with interleukin-2. Twenty of 107 (19%) patients developed sepsis and *S. aureus* was the causative organism in the majority of cases (13/20). Carriage of *S. aureus* increased the risk of *S. aureus* bacteremia 6.3-fold. Desquamation of the skin at the catheter insertion site increased the risk 2.0-fold. If both desquamation of the skin and carriage of *S. aureus* were present a relative risk for *S. aureus* bacteremia of 14.5 (95% confidence interval, 4.1 to 50.9) was found. Thus, in this specific population with an extremely high rate of bacteremia the role of carriage has been clearly established. In general, insertion of intravenous devices is not associated with such a high rate of bacteremia but it is likely that carriers of *S. aureus* will have a higher rate of *S. aureus* bacteremia. Pujol *et al.*[156] looked at bacteremia in an intensive care unit. Most of the *S. aureus* bacteremias were caused by an intravascular device. In this study carriers of *S. aureus* had a relative risk of 12.4 for the development of *S. aureus* bacteremia (Table 4). Again, this correlation should be investigated in further studies because it may have important implications for prevention.

Wegener granulomatosis. Wegener granulomatosis is a systemic disease characterized by necrotizing granulomatous inflammation of the upper and lower respiratory tract in combination with vasculitis and focal necrotizing crescentic glomerulonephritis.[53] Treatment with cyclophosphamide and corticosteroids has proven to be successful. After remission is achieved the clinical course is highly variable and relapses occur in most patients at variable intervals. Stegeman *et al.*[183] have recently reported a significant association between nasal carriage of *S. aureus* and a higher relapse rate of disease (Table 4). Also some anecdotal reports have stated that treatment with sulfamethoxazole-trimethoprim had a beneficial effect on the course of the disease,[195] which could be due to the reduction of *S. aureus* carriage by the antibiotic treatment. The underlying pathophysiological mechanisms remain to be elucidated and the effects of elimination of nasal carriage on the relapse rate should be studied more specifically.

MRSA. The difference between MRSA and methicillin-susceptible *S. aureus* (MSSA) is resistance to β-lactamase-stable β-lactam antibiotics. Often this is associated with resistance to multiple other antibiotics, which limits the therapeutic options. MRSA has become an important pathogen in many hospitals. In the United States, the proportion of MRSA has rapidly increased over the last decennium, being 29% in 1991.[143] In this report it was also shown that the prevalence generally increased with the size of the hospital. In Europe an interesting variation in the geographic distribution of MRSA is found. The prevalence of MRSA increases significantly from northern countries to the countries in the south.[204] The fact that carriage of MRSA poses an increased risk of infection over the risk of carriage of MSSA has been suggested by several authors. In CAPD patients, Lye *et al.*[111] found that MRSA carriers were at increased risk for MRSA infection compared with non-carriers (Table 4). Comparing MRSA carriers with MSSA carriers, they found a higher rate of peritonitis and exitsite infection in MRSA carriers. Moreover, in the group of MRSA carriers there was a significantly higher number of catheter losses and CAPD patient dropouts. Muder *et al.*[129] studied the consequences of MRSA carriage in a long-term care facility. MRSA carriers were at increased risk for the development of *S. aureus* infections. The occurrence of *S. aureus* infections in MSSA carriers was comparable to that of non-carriers. Mest *et al.*[121] found that perioperative colonization with MRSA significantly increased the risk for postoperative MRSA infection in patients on the intensive care unit. Pujol *et al.*[156] studied the rate of bacteremia in patients admitted to the intensive care unit. Patients colonized with MSSA in the nares were at a significantly increased risk for the development of *S. aureus* bacteremia (RR: 5.4, 95%

CI: 1.9 to 15.2 compared with non-carriers), but patients colonized with MRSA were at a much higher risk (RR: 21,7, 95% CI: 9.2 to 50.8 compared with non-carriers). The risk for MRSA carriers was significantly higher than the risk for MSSA carriers (RR: 4.0, 95% CI: 1.9 to 8.3). After adjusting for other predictors of bacteremia the relative risk for *S. aureus* bacteremia was 3.9 with a 95% confidence interval from 1.6 to 9.8 for MRSA carriers compared with MSSA carriers. The conclusion of these studies was that MRSA carriage constitutes a greater risk for the development of *S. aureus* infection than MSSA carriage. This could be a result of the resistance itself, of an increased intrinsic virulence of MRSA compared with MSSA, or of a more vulnerable category of patients being colonized by MRSA. A number of studies have failed to show that the virulence of MRSA differed from that of MSSA. These studies involved the in vitro production of extracellular hemolysins, enzymes, or toxins[147], intra leukocyte survival, or phagocytic destruction[147,202] as well as animal lethality studies.[76,147] Also, a clinical evaluation comparing the outcome of MRSA- with MSSA-infections did not reveal significant differences.[75] Since these studies indicate that there is no increased virulence of MRSA over MSSA it is most likely that the increased infection rate observed in carriers of MRSA is primarily due to the selection of the population of patients who become carriers of MRSA. In view of the rapid increase of the prevalence of MRSA and the problems associated with its control and therapy, more insight is needed into the epidemiology of MRSA colonization and infection to develop more effective, preventive strategies.

THE PATHOGENESIS OF ENDOGENOUS INFECTION

The nose is regarded as the major site of *S. aureus* carriage from where the organisms can spread to other parts of the body.[209,211] Reagan *et al.*[158] have shown that elimination of nasal carriage by using topical mupirocin also eliminates hand carriage. From these observations it can be concluded that the nose provides an environment in which *S. aureus* can propagate and maintain itself for prolonged periods. The proposed pathogenesis for a number of endogenous infections would be that from the nose, the skin becomes colonized which causes subsequent infection in patients with impaired skin sites; e.g., in hemodialysis[21] and CAPD patients, and in patients with intravascular catheters. For surgical patients there are other possibilities to be considered. First, most patients are intubated prior to surgery, traumatizing the epithelial lining of the throat which may cause hematogenous spreading of *S. aureus* to the surgical site. However, many surgical procedures include the use of antimicrobial prophylaxis which should protect against this route of infection. Another possibility is that *S. aureus* is dispersed from the nose into the air of the operating room and then contaminates the surgical site during surgery. This route of transmission could certainly have played a role in the early days of surgery when the air conditions in the operating room were not as well controlled as at present. A recent report on endogenous infection as a major cause of surgical wound infections[90] was conducted in an operating theatre with a laminar downflow unit positioned directly over the patient. Therefore, this route is not considered likely in the setting of the modern operating room. Finally, skin carriage of *S. aureus* in patients who are nasal carriers could be an explanation for endogenous infection. In nasal carriers the skin is often colonized by *S. aureus*. Preoperative disinfection may not be effective in the deeper layers of the skin and, thereby, *S. aureus* becomes a source of infection during surgery. It should be emphasized that these are only hypotheses on the pathogenetic mechanisms of *S. aureus* infection, all of which should be confirmed or refuted in further studies. These studies are needed because an optimal preventive strategy can be developed only when the pathogenesis is fully understood.

EFFECTS OF ELIMINATION OF CARRIAGE ON THE INFECTION RATES

Elimination strategies. In populations in which *S. aureus* nasal carriage is identified as a risk factor for infection it is conceivable that elimination of carriage would reduce the infection rate. Three approaches for elimination of carriage are available. First, the local application of antibiotics or disinfectants. Most often used are nasal ointments or sprays, sometimes combined with the application of disinfecting agents to the skin. In general, the results have been disappointing. Both a low efficacy and a rapid emergence of resistance to the agents used was observed. Results of such studies have been reviewed by others.[31,130,211] Recently, mupirocin (Bactroban, SmithKline Beecham Pharmaceuticals, Philadelphia, USA), a new antibiotic has become available for topical use. This agent has been shown to posses excellent efficacy for the elimination of *S. aureus* carriage and, therefore, has offered a new opportunity to eliminate *S. aureus* nasal carriage. Mupirocin is well tolerated and, when used appropriately (application to the nose b.i.d. for 5 days) development of resistance has not been reported. The results from a limited number of examplifying studies are reported here. A more extensive review of the literature on mupirocin has been published recently by Hudson.[81] In a randomized, double-blind place bo controlled multicenter study, Doebbeling *et al.*[41] found that when mupirocin was applied to the nose twice daily for 5 consecutive days, this resulted in elimination of carriage in 91% of stable nasal carriers. Four weeks post-treatment, 87% of the subjects remained free of nasal carriage. In the placebo group the post-treatment elimination rates were 6% and 7% respectively ($P<0.0001$). A subgroup was followed up for one year to determine the long-term efficacy of mupirocin. [42] At six months the nasal carriage rate in the treated group was 48% and in the placebo group 72% ($P=0.054$) and at 12 months the rates were 53 and 76%, respectively ($P=0.056$). Hand carriage at six months was significantly reduced in the treated group relative to controls (15% and 48%; $P=0.04$). The recolonizing strains were subjected to plasmid typing. Thirty-six percent were recolonized with a new strain at one year, whereas 34% had the original strain. Resistance to mupirocin was not observed. Comparable results were found by Fernandez *et al.*[54] In the studies mentioned above mupirocin was applied to healthy volunteers (health care workers). In patients on hemodialysis mupirocin was less effective.[24] A reduction from 90 to 33% directly after treatment and to 66% after four months was observed. Apparantly, in this group of patients other sites exist were *S. aureus* can maintain itself. The question remains if recolonization by an identical strain is the result of inadequate treatment with mupirocin. This would mean that the microorganism is able to persist on other sites of the body. Another possibility is that succesfully treated subjects are recolonized from external sources, which would not be considered a treatment failure. Although development of resistance to mupirocin was not observed in clinical studies for eradication of carriage it has been reported repeatedly in the literature. Generally, it was found in cases of prolonged use of the skin-preparation.[81] Resistance consists of two types, a low level and a high level resistance. The low-level form (MIC from 8-256 mg/L) consists of modifications in the target enzyme. The high level form (MIC >500 mg/L) consists of a mupirocin-insusceptible, plasmid-encoded enzyme.[127] This transmissible mechanism causes concern about the future spread of mupirocin resistance, when it is used on a large scale. Therefore restricted usage of this valuable and unique antimicrobial agent is recommended, in appropriately selected patients and for short courses only.

A second approach to eliminating nasal carriage is administration of systemic antibiotics. The results have been disappointing for most agents. To date only rifampicin has proven to be an effective agent, but side effects and the rapid emergence of resistant strains have limited its use for this purpose. Chow and Yu have given a comprehensive review on this subject.[31]

The third strategy is bacterial interference. That is active colonization with a strain of *S.*

aureus (502A) which is considered to posses minimal pathogenic properties but is able to prevent colonization by more virulent strains, presumably by competition for the binding sites in the nose. However, the exact mechanism for this effect has never been elucidated.[1] Interference was used successfully in nurseries during outbreaks of *S. aureus* infections in the 1960s and to treat patients with recurrent furunculosis.[25,103,175,185] However, this approach was occasionally complicated by serious infections due to *S. aureus* 502A[18,44] and even a fatal infection was reported.[78] Although the report documenting a fatal outcome concluded that "The benefits of *S. aureus* 502A programs far outweigh their hazards", this strategy was not pursued further at that time. In conclusion most strategies to eliminate carriage of *S. aureus* have been disappointing. Mupirocin has offered a new opportunity for this purpose and is considered by far the most effective agent available at this time.

Surgery. The concept of elimination of *S. aureus* carriage has been studied in surgical patients when the risk of carriage became evident. These early attempts were generally hampered by a lack of effective elimination methods available at that time. In a report by Gould and Allan in 1954[64] a nasal ointment containing tetracycline was used to treat nasal carriers among hospital staff members. After this intervention, a reduction of the *S. aureus* infection rate was observed, which was due to a reduction in the number of infections with "hospital staphylococci". When the carriage rate among staff members increased after the topical therapy was stopped the number of infections with hospital staphylococci also increased. In 1959 Weinstein[203] reported a study in patients who underwent chest surgery. The mean length of preoperative hospitalization in this group was extremely long (4 months). Most patients were treated for tuberculosis. Nose swabs were taken when surgery was being planned. When *S. aureus* was cultured, topical treatment of the nose with bacitracin-neomycin ointment t.i.d. was started and continued until the fourth to fifth postoperative day. The overall infection rate in carriers was significantly higher despite the treatment. However, these results are difficult to interpret because of the high failure rate of the elimination therapy. Nineteen carriers who were treated adequately and had follow-up cultures taken, were considered evaluable. Twelve of them converted to negative nasal cultures, seven remained positive. The infection rate in the unsuccessfully treated carriers was much higher (5/7) than in the succesfully treated carriers (0/12). Although the numbers are small, these findings suggest a beneficial effect of elimination of nasal carriage on the infection rate.

The first double-blind, placebo controlled trial of nasal disinfection using naseptin cream was reported by Henderson and Williams in 1961.[73] They observed no effect on the postoperative *S. aureus* wound infection rate in 850 patients (5.0% among treated and 4.6% among placebo patients). Again in this study, a high failure rate of elimination of *S. aureus* nasal carriage was found. Another outcome in this study was a higher *S. aureus* infection rate in the non-carriers in the treated group which approached statistical significance (4.6% versus 1.1%, P=0.07). A similar trial was performed by Stokes and Milne.[184] They found a *S. aureus* infection rate of 12 (3.9%) of 308 treated patients compared with 16 (5.6%) of 285 placebo patients (P=0.34). Also the infection rate in non-carriers who were treated was higher than in non-carriers who were not treated (4/207 versus 2/193 (P=0,69)) and the failure rate of successful eradication of carriage was high. Rountree *et al.*[163] also used naseptin but did not use a placebo in the control group. They found a significant reduction of the *S. aureus* infection rate in the treated group (3 (3.5%) of 84 versus 16 (16.0%) of 99, (P=0.007)). The differences in the effects observed in these studies may be caused by differences in the local epidemiology of *S. aureus* infections, such as the presence of environmental reservoirs not influenced by elimination of nasal carriage. The major drawback of these studies, however, is the poor efficacy of the treatments used to achieve the primary target, i.e. elimination of nasal carriage of *S. aureus*.

As stated above this problem of efficacy is now largely overcome by the introduction of

mupirocin ointment in the late 1980s. Using mupirocin as a perioperative prophylaxis in a surgical population where the risk factor of nasal carriage was clearly established, a highly significant reduction of the surgical wound infection rate was found.[91] All patients underwent thoracic surgery in the same department and were under prospective surveillance for the development of infections. An historical control group (n=928) was compared with an intention-to-treat group (n=868) of which 752 were actually treated. The surgical wound infection rate in the control group was 7.3% and was 2.8% in the intention-to-treat group (P<0.0001). The reduction was even stronger in the treated group (1.7%). In the not-treated group after the intervention, the rate (7.4%) was comparable to the control group. These results look very promising and await confirmation in a randomized, placebo controlled, double blind trial.

Hemodialysis. Several oral and topical antibiotics have been studied for eradication of *S. aureus* nasal carriage in hemodialysis patients. These studies were summarized by Chow and Yu.[31] Rifampicin has been the most effective oral agent used for this purpose. Yu *et al.*[217] used rifampicin in conjunction with nasal bacitracin and obtained a significant reduction of the *S. aureus* infection rate in their population of hemodialysis patients. However, a rapid emergence of rifampicin-resistant strains was observed. Mupirocin has been evaluated in hemodialysis in 6 studies reviewed by Boelaert.[21] Using a short term course of therapy of five to ten days, a high elimination rate immediately post-therapy was found (average 87%, range 76 to 100%). However, in some studies the nares were sampled at three months post-therapy and a relapse rate of 20 to 77% was found. Therefore, a schedule of continuous mupirocin was proposed by Boelaert *et al.*[22] In a randomized, double-blind placebo controlled trial, they treated stable nasal carriers with mupirocin for two weeks t.i.d., and then thrice weekly for a total of 9 months. A highly significant reduction in the carriage rate of the treated group (only 6% of the cultures were positive) was observed, accompanied by a significant reduction in the *S. aureus* infection rate (1/104 patient months among treated and 6/147 patient months among not-treated (P<0.05)). The administration of mupirocin to nasal carriers was later adjusted to an initial course of 5 days t.i.d., and thereafter once a week during the remaining period on hemodialysis. Using this schedule a highly effective elimination of carriage was achieved and this was accompanied by a four to six-fold reduction in the *S. aureus*-bacteremia rate.[23,93] Although resistance to mupirocin was observed in only one patient,[23] it is a point of concern with these prolonged treatment schedules. In conclusion, elimination of nasal carriage in hemodialysis patients using mupirocin significantly reduces the *S. aureus* infection rates.

Bloom *et al.*[19] performed a cost-effectiveness analysis of mupirocin in hemodialysis. Two preventive strategies were compared. First, screen all patients by a nasal culture every three months and treat those with *S. aureus* with mupirocin for 5 days b.i.d (Strategy 1). The second strategy was to treat all patients irrespective of their carrier state with mupirocin weekly for 3 days b.i.d (Strategy 2). These strategies were compared with eachother and with no mupirocin applications, called treat infection only. The annual savings of strategy 1 per 1000 dialysis patients were $784,000 and of strategy 2 the savings were $1,117,000. Also both preventive strategies prevented death and improved the quality of life. Although, from this analysis it could be concluded that the treat all strategy is preferrable, the authors comment that the risk of development of resistance with such a widespread use of mupirocin is increased. Therefore, strategy 2 may be a better choice. Further studies are needed to determine which strategy gives maximal efficacy and minimal development of resistance.

CAPD. Zimmerman *et al.*[219] studied the effects of intermittent administration of rifampicin in patients on CAPD in a randomized controlled trial. Sixty-four patients were randomized, irrespective of their carrier state, to receive rifampicin 300 mg b.i.d. for five days which was repeated every three months or to receive nothing. No significant differences between the *S.*

aureus colonization rates were observed. Also, there was no significant difference in the *S. aureus* peritonitis rates. In the treated group a statistically significant reduction was observed in the time to the first catheter-related infection and the catheter infection rate (both those caused by *S. aureus* and the overall rate). However, in the treated group, resistance to rifampicin emerged in four patients compared with none of the control patients. Dryden *et al.*[45] found a reduction of the infection rate after an intervention program which included elimination of nasal carriage and stringent aseptic care of the exit site. Because a number of measures was taken at the same time the effect of elimination of nasal carriage could not be established. Until now two reports have been published studying the effects of mupirocin on the infection rate in CAPD patients. Perez-Fontan *et al.*[148] used an historical control group and compared it with the results from a group that was cultured three times at monthly intervals. If *S. aureus* was isolated, a seven day course with mupirocin t.i.d. was given. Seven days after this treatment and monthly thereafter a control culture was performed. If recolonization was found a new course of mupirocin was given. The initial efficacy was 100%, but recolonization occurred frequently, especially after three months. The *S. aureus* peritonitis rate was significantly reduced but the overall peritonitis rate was not, mainly due to a significantly higher rate of peritonitis caused by gram-negative bacteria in the treated group compared to the not-treated group. Similar observations were made for catheter exit-site infections. Due to the severity of *S. aureus* catheter exit-site infections, there was a significant lower catheter loss due to exit-site infections in the treated group. During the two-year study period a gradual increase of resistance among *S. aureus* isolates to mupirocin was observed which gives great concern for the future developments in this setting. Although a historical control group was used, the reduction in *S. aureus* infections is likely to be caused by the application of mupirocin. However, the unexplained observation that the rate of infection due to other microorganisms was increased makes these results difficult to interpret. Therefore a randomized, placebo controlled double blind multi-center study was performed.[52] Nasal carriers were treated with mupirocin or placebo ointment twice daily for five days. This was repeated every four weeks. In 1,144 patients screened, 267 carriers were identified (23.3%). The *S. aureus* exit-site infection rate was significantly lower in the treated group (1 in 99.3 patient months versus 1 in 28.1 patient months, P=0.006). There was no significant increase in gram-negative infections and development of resistance to mupirocin was not observed. Why resistance was observed in the previous study and not in this one is not clear. Nevertheless, the possibility of development of resistance should be taken seriously when using mupirocin for prolonged periods such as in CAPD patients. It can be concluded that elimination of *S. aureus* nasal carriage in patients on CAPD decreases the exit site infection rate. The effect on the peritonitis rates remains unclear.

MRSA. In their consensus review Mulligan *et al.*[130] state that indications for eradication of MRSA are to stop an outbreak in a health-care setting, or to prevent recurrent infections in an individual. In settings where MRSA is endemic, elimination of carriage has not been found to be cost-effective and is, therefore, considered not indicated by these authors. In an outbreak situation, the first goal should be to identify all carriers, including patients and health-care workers. Then elimination of carriage should be achieved in all so identified.[32,92,130] Most reports on the effects of elimination of MRSA carriage in outbreak situations also mention that other infection control measures were taken concomitantly. These other interventions may have played a significant role to control these outbreaks. One report mentioned the complete control of an outbreak when only simple infection control measures were taken,[68] while several others found that only extensive modification of local infection control practices were effective[17,26,92,132] Therefore, infection control measures should be accompanied by identification of carriers and, subsequently, elimination of *S. aureus* carriage.[215] The effects of

elimination strategies in settings were MRSA is endemic have not been studied extensively. One study in a surgical intensive care unit reported a significant decrease of the colonization and pulmonary tract infection rate when all patients were treated during the first week after admittance b.i.d.[163]

GENERAL CONCLUSIONS

"Among the more chastening chapters in the annals of microbiological research is the story of our apparently dismal failure to control the depredations of the staphylococcus. Repeatedly, fresh light has been shed upon the habits and habitats of these minute clustered spherules, and once outmoded hypotheses about their metabolic mechanisms have been refurbished. Yet three quarters of a century after Koch first noted their presence in pus, the staphylococci (like Francis Thompson's angels) "keep their ancient places", no less ubiquitous but still elusive, and (like Lucifer at least) shockingly endowed with apparantly new, malign propensities". This quote is from a manuscript describing the first seven decades of staphylococcal research by Dolman, published in 1955.[43] Since then, four decades of extensive research have passed and it seems that nothing has changed really. New staphylococcal diseases have been recognized, like the toxic shock syndrom, and we are still unable to control the spread of staphylococci and the development of resistance. World-wide, MRSA-rates have increased dramatically during the last decades. The treat of development of resistance to vancomycin, the only antimicrobial agent effective against MRSA, is dooming. The worldwide use of vancomycin has increased dramatically over the last years. Concordantly resistance in other gram-positive micro-organisms has increased significantly. The resistance of enterococci in intensive care units (ICU's) of US hospitals may serve as an example. In 1989, <0.5% of enterococci were resistant. In 1992, resistance had increased to nearly 8%.[67] It has been shown both in vitro and in animal studies that high-level vancomycin resistance can easily be transferred from Enterococci to *S. aureus*.[139] Therefore, it seems inevitable that this will also happen in clinical settings. This totally-resistant microorganism would have dramatic impact on morbidity and mortality in the hospitalized patients. Optimalization of preventive strategies is needed to control staphylococci. Therefore new strategies have to be developed or, like Dolman stated, old methods have to be refurbished. The ability to control staphylococcal infections in the future will depend on many factors, e.g. the development of new therapeutic agents, optimalization of infection control measures, the introduction of new medical devices with a reduced risk of infection etc. In this review the importance of nasal carriage has been summarized. Nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* infections. It has been clearly established that it is a major risk factor for the development of infection in certain groups of patients (e.g. hemodialysis, CAPD, surgery, patients with intra-vascular devices and HIV). The underlying mechanisms of nasal carriage are as yet unknown. In view of the benefits to be expected from elimination of nasal carriage in groups at risk, the pathogenesis of endogenous infection has to be elucidated. Only then can the most effective elimination strategy be developed. For this moment mupirocin is the most effective drug available to achieve eradication of carriage. However, resistance to mupirocin is increasing and it has to be questioned for how long this unique agent will be effective. One strategy which has been used succesfully in the past is bacterial interference. This alternative approach to controlling staphylococcal infections could offer new opportunities if a strain with minimal virulence and maximal competion for the binding sites in the nose was developed.

REFERENCES

1 **Aly R, Maibach HI, Shinefield HR, Mandel A, Strauss WG.** Bacterial interference among strains of *Staphylococcus aureus* in man. *J Infect Dis* 1974;**129**:720-4.

2 **Aly R, Shinefield HR, Litz C, Maibach HI.** Role of teichoic acid in the binding of *Staphylococcus aureus* to nasal epithelial cells. *J Infect Dis* 1980;**141**:463-5.

3 **Aly R, Shinefield HR, Maibach HI.** *Staphylococcus aureus* adherence to nasal epithelial cells: studies of some parameters. In: "*Skin microbiology*" (Maibach and Aly, Eds.), Springer Verlag, New York. 1981;171-9

4 **Aly R, Maibach HI, Strauss WG, Shinefield HR.** Effects of systemic antibiotic on nasal bacterial etiology in man. *Applied Microbiol* 1970;**20**:240-4.

5 **Aly R, Shinefield HR, Strauss WG, Maibach HI.** Bacterial adherence to nasal mucosal cells. *Infect Immunity* 1977;**17**:546-9.

6 **Aly R, Shinefield HI, Strauss WG, Maibach HI.** Bacterial adherence to nasal mucosal cells. *Infect Immun* 1977;**17**:546-9.

7 **Aly R, Maibach HI, Shinefield HR.** Microbial flora of atopic dermatitis. *Arch Dermatol* 1977;**113**:780-2.

8 **Aly R, Maibach HI, Shinefield HR, Mandel A.** *Staphylococcus aureus* carriage in twins. *Am J Dis Child* 1974;**127**:486-8.

9 **Aly R, Levit S.** Adherence of *Staphylococcus aureus* to squamous epithelium: role of fibronectin and teichoic acid. *Rev Infect Dis* 1987;**9**:341-50.

10 **Amir M, Paul J, Batchelor B, Kariuki S, Ojoo J, Waiyaki P, Gilks C.** Nasopharyngeal carriage of *Staphylococcus aureus* and carriage of tetracyclin-resistant strains associated with HIV-seropositivity. *Eur J Clin Microbiol Infect Dis* 1995;**14**:34-40.

11 **Armstrong-Esther CA, Smith JE.** Carriage patterns of *Staphylococcus aureus* in a healthy non-hospital population of adults and children. *Ann Hum Biol* 1976;**3**:221-7.

12 **Bassett HFM, Ferguson WG, Hoffman E, Walton M, Blowers R, Conn CA.** Sources of staphylococcal infection in surgical wound sepsis. *J Hyg (Camb)* 1963;**61**:83-94.

13 **Battan R, Raviglione MC, Wallace J, Cort S, Boyle JF, Taranta A.** *S. aureus* nasal carriage among homosexual men with and without HIV infection. *Am J Infect Control* 1991;**19**:98-100.

14 **Bergqvist S.** Observations concerning the presence of pyogenic staphylococci in the nose and their relationship to the antistapholysin titre. *Acta Med Scand* 1950;**136**:343-50.

15 **Berman DS, Schaefler S, Simberkoff MS.** *Staphylococcus aureus* colonization in intravenous drug abusers, dialysis patients and diabetes. *J Infect Dis* 1987;**155**:829-31.

16 **Biesbrock AR, Reddy MS, Levine MJ.** Interaction of a salivary mucin secretory immunoglobulin A complex with mucosal pathogens. *Infect Immun* 1991;**59**:3492-7.

17 **Bitar CM, Mayhall CG, Lamb VA, Bradshaw TJ, Spadora AC and Dalton HP.** Outbreak due to methicillin- and rifampicin-resistant *Staphylococcus aureus*: Epidemiology and eradication of the resistant strain from the hospital. *Infect Control* 1987;**8**:15-23.

18 **Blair EB, Tull AH.** Multiple infections among newborns resulting from colonization with *Staphylococcus aureus* 502A. *Am J Clin Path* 1969;**52**:42-9.

19 **Bloom BS, Fendrick AM, Chernew ME, Patel P.** Clinical and economic effects of mupirocin calcium on preventing *Staphylococcus aureus* infection in hemodialysis patients: a decision analysis. *Am J Kid Dis* 1996;**27**:687-94.

20 **Blumberg EA, Hatcher VB, Lowy FD.** Acidic fibroblast growth factor modulates *Staphylococcus aureus* adherence to human endothelial cells. *Infect Immun* 1988;**56**:1470-4.

21 **Boelaert JR.** *Staphylococcus aureus* infection in hemodialysis patients. Mupirocin as a topical strategy against nasal carriage: a review. *J Chemother* 1994;**Suppl 2**:19-24.

22 **Boelaert JR, DeSmedt RA, De Baere YA, Godard CA, Matthys EG, Schurgers ML, Daneels RF, Gordts BZ, Van Landuyt HW.** The influence of calcium mupirocin nasal ointment on the incidence of *Staphylococcus aureus* infections in hemodialysis patients. *Nephrol Dial Transplant* 1989;**4**:278-281.

23 **Boelaert JR, Van Landuyt HW, Godard CA *et al.*** Nasal mupirocin ointment decreases the incidence of *Staphylococcus aureus* bacteremias in hemodialysis patients. *Nephrol Dial Transplant* 1993;**8**:235-9.

24 **Bommer J, Vergetis W, Andrassy K, Hingst V, Borneff M, Huber W.** Elimination of *Staphylococcus aureus* in hemodialysis patients. *ASAIO J* 1995;**41**:127-31.

25 **Boris M, Seller TF, Eichenwald HF, Ribble JC, Shinefield HR.** Bacterial interference. *Am J Dis Child* 1964;**108**:252-61.

26 **Boyce JM, Landry M, Deetz TR, DuPont HL.** Epidemiologic studies of an outbreak of nosocomial methicillin-resistant *Staphylococcus aureus* infections. *Infect Control* 1981;**2**:110-6.

27 **Boyko EJ, Lipsky BA, Sandoval *et al.*** NIDDM and prevalence of nasal *Staphylococcus aureus* colonization: San Luis Valley diabetes study. *Diabetes Care* 1989;**12**:189-92.

28 **Bruun JN.** Postoperative wound infection. Predisposing factors and the effect of a reduction in the dissemination of staphylococci. *Acta Med Scand* 1970;**514 (Suppl)**:1-89.

29 **Calia FM, Wolinsky E, Mortimer EA, Abram JS, Rammelkamp CH.** Importance of the carrier state as a source of *Staphylococcus aureus* in wound sepsis. *J Hyg Camb* 1969;**67**:49-57.

30 **Carruthers MM, Kabat WJ.** Mediation of staphylococcal adherence to mucosal cells by lipoteichoic acid. *Infect Immun* 1983;**40**:444-6.

31 **Chow JW, Yu VL.** *Staphylococcus aureus* nasal carriage in hemodialysis patients. Its role in infection and approaches to prophylaxis. *Arch Int Med* 1989;**149**:1258-62.

32 **Coello R, Garcia M, Arroyo P, Minguez D, Fernandez C, Cruzet F, Gaspar C.** Prospective study of infection, colonization and carriage of methicillin-resistant *Staphylococcus aureus* in an outbreak affecting 990 patients. *Eur J Clin Microbiol Infect Dis* 1994;**13**:74-81.

33 **Colbeck JC, Robertson HR, Sutherland WH and Hartley FC.** The importance of endogenous staphylococcal infections in surgical patients. *Canad Serv Med J* 1959;**15**:326-31.

34 **Cookson BD, Aparicio P, Deplano A, Struelens M, Goering R, Marples R.** Inter-centre comparison of pulsed field gel electrophoresis for the typing of methicicllin-resistant *Staphylococcus aureus*. *J Med Microbiol* 1996;**44**:179-84.

35 **Cree RGA, Aleljung P, Paulsson M, Witte W, Noble WC, Ljungh A, Wadstrom T.** Cell surface hydrophobicity and adherence to extra-cellular matrix proteins in two collections of methicicllin resistant *Staphylococcus aureus*. *Epidemiol Infect* 1994;**122**: 307-14.

36 **Crossley K, Landesmann B, Zaske D.** An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. *J Infect Dis* 1979;**139**:280-7.

37 **Dan M, Moses Y, Poch F, Asherov J, Gutman R.** Carriage of methicillin-resistant *Staphylococcus aureus* by non-hospitalized subjects in Israel. *Infection* 1992;**20**:332-5.

38 **Dancer SJ, Noble WC.** Nasal, axillary, and perineal carriage of *Staphylococcus aureus* among women: identification of strains producing epidermolytic toxin. *J Clin Path* 1991;**44**:681-4.

39 **Davies SJ, Ogg CS, Cameron JS, Poston S, Noble WC.** *Staphylococcus aureus* nasal carriage, exit-site infections and catheter loss in patients treated with continuous ambulatory peritoneal dialysis (CAPD). *Peritoneal Dial Int* 1989;**9**:61-4.

40 **Decker MD, Lybarger JA, Vaughn WK, Hutcheson RH, Schaffner W.** An outbreak of staphylococcal skin infections among river rafting guides. *Am J Epidemiol* 1986;**124**:969-76.

41 **Doebbeling BN, Breneman DL, Neu HC, Aly R, *et al.*** Elimination of *Staphylococcus aureus* nasal carriage in health care workers: analysis of six clinical trials with calcium mupirocin ointment. *Clin Infect Dis* 1993;**17**:466-74.

42 **Doebbeling BN, Reagan DR, Pfaller MA, Houston AK, Hollis RJ, Wenzel RP.** Long-term efficacy of intranasal mupirocin ointment. *Arch Intern Med* 1994;**154**:1505-8.

43 **Dolman CE.** The staphylococcus: seven decades of research (1855-1955). *Can J Microbiol* 1955;**2**:189-200.

44 **Drutz DJ, Van Way MH, Schaffner W, Glenn Koenig M.** Bacterial interference in the therapy of recurrent staphylococcal infections: multiple abcesses due to the implantation of the 502A strain of *Staphylococcus*. *New Engl J Med* 1966;**275**:1161-5.

45 **Dryden MS, Ludlam HA, Wing AJ, Phillips I.** Active intervention dramatically reduces CAPD-associated infection. *Adv Perit Dial* 1991;**7**:125-8.

46 **Duncan IBR, Collins AM, Neelin EM, Roy TE.** Nasal carriage of *Staphylococcus pyogenes* by student nurses. *Can Med Ass J* 1957;**Dec**:1001-9.

47 **Ehrenkranz NJ.** Nasal rejection of experimentally inoculated *Staphylococcus aureus*: evidence for an immune reaction in man. *J Immunol* 1966;**96**:509-17.

48 **Ehrenkranz NJ.** Person-to-person transmission of *Staphylococcus aureus*: Quantitative characterization of nasal carriers spreading infection. *N Engl J Med* 1964;**271**:225-30.

49 **Ena J, Boelaert JR, Boyken LD, Van Landuyt HW, Godard CA, Herwaldt LA.** Epidemiology of *Staphylococcus aureus* infections in patients on hemodialysis. *Infect Cont Hosp Epidemiol* 1994;**15**:78-81.

50 **Espersen F, Clemmensen I.** Isolation of a fibronectin-binding protein from *Staphylococcus aureus*. *Infect Immun* 1982;**37**:526-31.

51 **Fainstein V, Musher DM, Cate TR.** Bacterial adherence to pharyngeal cells during viral infection. *J Infect Dis* 1980;**141**:172-6.

52 **Faller B.** New findings on the prevention of exit site infections. European multicenter trial results. 15th annual conference on peritoneal dialysis. February 12-14, 1995. Baltimore, Maryland.

53 **Fauci AS, Haynes BF, and Katz P.** The spectrum of vasculitis. Clinical, pathological, immunologic and therapeutic considerations. *Ann Intern Med* 1978;**89**:660-76.

54 **Fernandez C, Gaspar C, Torrellas A, Vindel A, Saez-Nito JA, Cruzet F, Aguilar L.** A double-blind, randomized, placebo-controlled clinical trial to evaluate the safety and efficacy of mupirocin calcium ointment for eliminating nasal carriage of *Staphylococcus aureus* among hospital personnel. *J Antimicrob Chemother* 1995;**35**:399-408.

55 **Findlay GM, Abrahams C.** The incidence of staphylococci in the nose and on the skin of Africans and Europeans in West-Africa. *J Roy Army Med Corps* 1946;**87**:272-4.

56 **Foster TJ, McDevitt D.** Surface-associated proteins of *Staphylococcus aureus*: their possible role in virulence. *FEMS Microbiol Let* 1994;**118**:199-206.

57 **Frenay, HM, Theelen JPG, Schouls LM, Vandenbroucke-Grauls CMJE, Verhoef J, van Leeuwen WJ, Mooi FR.** Discrimination of epidemic and non-epidemic strains of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphisms. *J Clin Microbiol* 1994;**32**:846-7.

58 **Frost AJ, Wanasinghe DD, Woolcock JB.** Some factors affecting selective adherence of microorganisms in the bovine mammary gland. *Infect Immun* 1977;**15**:245-53.

59 **Ganesh R, Castle D, McGibbon D, Phillips I, Bradbeer C.** Staphylococcal carriage and HIV infection. *Lancet* 1989;**Sep**:558.

60 **Gittelman PD, Jacobs JB, Lebowitz AS, Tierno PM.** *Staphylococcus aureus* nasal carriage in patients with rhinosinusitis. *Laryngoscope* 1991;**101**:733-7.

61 **Goldblum SE, Reed WP, Ulrich JA, Goldman RS.** Staphylococcal carriage and infections in hemodialysis patients. *Dial Transplant* 1978;**7**:1140-63.

62 **Goslings WRO, Büchli K.** Nasal carriage rate of antibiotic-resistant staphylococci. *Arch Intern Med* 1958;**102**:691-715.

63 **Gould D, Ramirez-Ronda CH, Holmes RK, Sanford JP.** Adherence of bacteria to heart valves in vitro. *J Clin Invest* 1975;**56**:1364-70.

64 **Gould JC, Allan WSA.** *Staphylococcus pyogenes* crossinfection: prevention by treatment of carriers. *Lancet* 1954;**2**:988-9.

65 **Gould JC, Cruikshank JD.** Staphylococcal infections in general practice. *Lancet* 1957;**2**:1157-61.

66 **Gould JC, McKillop E.** The carriage of *Staphylococcus pyogenes* var. *aureus* in the human nose. *J Hyg (Camb)* 1954;**52**:304-10.

67 **Grace Emori T, Gaynes RP.** An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev* 1993;**6**:428-44.

68 **Guiguet M, Rekacewicz C, Leclerq B, Brun Y, Escudier B and Andermont A.** Effectiveness of simple measures to control an outbreak of nosocomial methicillin-resistant *Staphylococcus aureus* in an intensive care unit. *Infect Control Hosp Epidemiol* 1990;**11**:23-6.

69 **Haagen IA, Heezius HC, Verkooyen RP, Verhoef J, Verbrugh HA.** Adherence of peritonitis-causing staphylococci to human peritoneal mesothelial cell monolayers. *J Infect Dis* 1990;**161**:266-73.

70 **Hallman FA.** Pathogenic staphylococci in the anterior nares: their incidence and differentiation. *Proc Soc Exp Biol Med* 1937;**36**:789-94.

71 **Hare R, Thomas CG.** The transmission of *Staphylococcus aureus*. *Br Med J* 1956;**2**:840-4.

72 **Hedstrom SA.** Recurrent staphylococcal furunculosis. Bacteriological findings and epidemiology in 100 cases. *Scand J Infect Dis* 1981;**13**:115-9.

73 **Henderson RJ, Williams REO.** Nasal disinfection in prevention of post-operative staphylococcal infection of wounds. *Brit Med J* 1961;**2**:330-3.

74 **Henderson RJ, Williams REO.** Nasal carriage of staphylococci and postoperative staphylococcal wound infection. *J Clin Path* 1963;**16**:452.

75 **Hershow RC, Khayr WF, Smith NL.** A comparison of clinical virulence of nosocomially acquired methicillin-resistant and methicillin-sensistive *Staphylococcus aureus* infections in a university hospital. *Infect Cont Hosp Epidemiol* 1992;**13**:587-93.

76 **Hewitt H, Sanderson PJ.** The effect of methicillin on skin lesions in guinea pigs caused by "methicillin-sensitive" and "methicillin-resistant" *Staphylococcus aureus*. *J Med Microbiol* 1974;**7**:223-8.

77 **Hoeksma A, Winkler KC.** The normal flora in the nose in twins. *Acta Leidensia* 1963;**32**:123-33.

78 **Houck PW, Nelson JD, Kay JL.** Fatal septicemia due to *Staphylococcus aureus* 502A. Report of a case and review of the infectious complications of bacterial interference programs. *Am J Dis Child* 1972;**123**:45-8.

79 **Howe CW.** Sources of postoperative staphylococcal infection. *Antimicrob Agents Chemother* 1963;**30**:671-8.

80 **Hu L, Umeda A, Kondo S, Amako K.** Typing of *Staphylococcus aureus* colonising human nasal carriers by pulsed field gel electrophoresis. *J Med Microbiol* 1995;**42**:127-32.

81 **Hudson IRB.** The efficacy of intranasal mupirocin in the prevention of Staphylococcal infections: A review of recent experience. *J Hosp infect* 1994;**27**:81-98.

82 **Imundo L, Barasch J, Prince A, Al-Awqati Q.** Cystic fibrosis epithelial cells have a receptor for pathogenic bacteria on their apical surface. *Proc Natl Acad Sci USA* 1995;**92**:3019-23.

83 **Jacobs SI, Williamson GM, Willis AT.** Nasal abnormality and the carrier rate of *Staphylococcus aureus*. *J Clin Path* 1961;**14**:519-21.

84 **Jacobson MA, Gellermann H, Chambers H.** *Staphylococcus aureus* bacteremia and recurrent staphylococcal infection in patients with AIDS and AIDS-related complex. *Am J Med* 1988;**85**:172-6.

85 **Kaplowitz LG, Comstock JA, Landwehr DM, Dalton HP, and Mayhall CG.** Prospective study on microbial colonization of the nose and skin and infection of the vascular access site in hemodialysis patients. *J Clin Microbiol* 1988;**26**:1257-62.

86 **Ketcham AS, Lieberman JA, West JT.** Antibiotic prophylaxis in cancer surgery and its value in staphylococcal carrier patients. *Surg Gynec Obstet* 1963;**117**:1-6.

87 **Kinsman OS, McKenna R, Noble WC.** Association between histocompatibility antigens (HLA) and nasal carriage of *Staphylococcus aureus*. *J Med Microbiol* 1983;**16**:215-20.

88 **Kirmani N, Tuazon CU, Murray HW, Parrish AE, Sheagren JN.** *Staphylococcus aureus* carriage rate of patients receiving long-term hemodialysis. *Arch Int Med* 1978;**138**:1657-9.

89 **Kirmani N, Tuazon CU, Alling D.** Carriage rate of Staphylocccus aureus among patients receiving allergy injections. *Ann Allergy* 1980;**45**:235-7.

90 **Kluytmans JAJW, Mouton JW, IJzerman EPF, Vandenbroucke-Grauls CMJE, Maat AWPM, Wagenvoort JHT, Verbrugh HA.** Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery. *J Infect Dis* 1995;**171**:216-9.

91 **Kluytmans JAJW, Mouton JM, VandenBergh MFQ, Manders MAAJ, Maat APWM, Wagenvoort JHT, Michel MF, Verbrugh HA.** Reduction of surgical wound infections in cardio-thoracic surgery by elimination of nasal carriage of *Staphylococcus aureus*. This thesis.

92 **Kluytmans JAJW, Leeuwen van W, Goessens W, Hollis R, Messer S, Herwaldt L, Bruining H, Heck M, Rost J, Leeuwen van N, Belkum van A and Verbrugh H.** Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and geno-typing. *J Clin Microbiol* 1995;**33**:1121-8.

93 **Kluytmans JAJW, Manders MAAJ, van Bommel EF, Verbrugh HA.** Prevention of *Staphylococcus aureus* bacteremia in hemodialysis patients by elimination of nasal carriage using mupirocin. This thesis.

94 **Kluytmans JAJW.** Unpublished results.

95 **Kluytmans JAJW, van Leeuwen WB, Goessens WHF, Hollis RA, Messer SA, Herwaldt LA, Bruining HA, Heck M, van Leeuwen N, Rose J, van Belkum A, and Verbrugh HA.** Food initiated outbreak of methicillin resistant *Staphylococcus aureus*, analyzed by pheno- and genotyping. *J Clin Microbiol.* 1995;**33**:1121-8.

96 **Kluytmans JAJW.** Surgical infections including burns. In: Wenzel RP, ed. *Prevention and control of nosocomial infections.* Williams and Wilkins, 3rd ed 1996. In Press.
97 **Krivan HC, Roberts DD, Ginsburg V.** Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence Nac β1-4 Gal found in some glycolipids. *Proc Natl Acad Sci USA* 1988;**85**:6157-61.
98 **Kropec A, Huebner J, Riffel M, Bayer U, Benzing A, Geiger K, Daschner FD.** Exogenous or endogenous reservoirs of nosocomial *Pseudomonas aeruginosa* or *Staphylococcus aureus* infections in a surgical intensive care unit. *Intensive Care Med* 1993;**19**:161-5.
99 **Lamikanra A, Paul BD, Akinwole OB, Paul MO.** Nasal carriage of *Staphylococcus aureus* in a population of healthy Nigerian students. *J Med Microbiol* 1985;**19**:211-6.
100 **Leedom JM, Kennedy RP, Lepper MH, Jackson GG, Dowling HF.** Observations of the staphylococcal nasal carrier state. *Ann N Y Acad Sci* 1965;**128**:381-403.
101 **Leigh DA, Joy G.** Treatment of familial Staphylococcal infection-comparison of mupirocin nasal ointment and chlorhexidin/neomycin (Naseptin) cream in eradication of nasal carriage. *J Antimicrob Chem* 1993;**31**:909-17.
102 **Lidwell OM, Polakoff S, Davies J, Hewitt JH, Shooter RA, Walker KA, Gaya H, Taylor GW.** Nasal acquisition of *Staphylococcus aureus* in a subdivided and mechanically ventilated ward: endemic prevalence of a single staphylococcal strain. *J Hyg Camb* 1970;**68**:417-33.
103 **Light IJ, Sutherland JM, Schott JE.** Control of a staphylococcal outbreak in a nursery: use of bacterial interference. *JAMA* 1965;**193**:699-704.
104 **Light IJ, Sutherland JM, Cochran ML, Sutorius J.** Ecologic relation between *Staphylococcus aureus* and *Pseudomonas* in a nursery population. Another example of bacterial interference. *New Engl J Med* 1968;**278**:1243-7.
105 **Lindahl M, Holmberg O, Jonsson P.** Adhesive proteins haemagglutinating *Staphylococcus aureus* isolated from bovine mastitis. *J Gen Microbiol* 1990;**136**:935-9.
106 **Lindbom G, Laurell G, Grenvick A.** Studies on the epidemiology of staphylococcal infections. *Acta Path Microbiol Scand* 1967;**69**:219-36.
107 **Lipsky BA, Pecoraro RE, Chen MS, Koepsell TD.** Factors affecting staphylococcal colonization among NIDDM outpatients. *Diabetes Care* 1987;**10**:483-6.
108 **Lipsky BA, Peugeot RL, Boyko EJ, Kent DL.** A prospective study on *Staphylococcus aureus* nasal colonization and intravenous therapy-related phlebitis. *Arch Intern Med* 1992;**152**:2109-12.
109 **Luzar MA.** Exit-site infection in continuous ambulatory peritoneal dialysis: a review. *Perit Dial Int* 1991;**11**:333-40.
110 **Luzar MA, Coles GA, Faller B, Slingeneyer A, Dah G, Briat C, Wone C, Knefati Y, Kessler M, Peluso F.** *Staphylococcus aureus* nasal carriage and infection in patients on continous ambulatory peritoneal dialysis. *N Engl J Med* 1990;**322**:505-9.
111 **Lye WC, Leong SO, Lee EJC.** Methicillin-resistant *Staphylococcus aureus* nasal carriage and infections in CAPD. *Kidney Int* 1993;**43**:1357-62.
112 **Mamo W, Fröman G.** Adhesion of *Staphylococcus aureus* to bovine mammary epithelial cells induced by growth in milk whey. *Microbiol Immunol* 1994;**38**:305-8.
113 **McAnally TP, Lewis MR, Brown DR.** Effect of rifampicin and bacitracin on nasal carriers of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1984;**25**:422-6.
114 **McKeown-Longo PJ.** Fibronectin-cell surface interactions. *Rev Infect Dis* 1987;**9(Suppl 4)**:S322.
115 **McNeely TB, Coonrod JD.** Comparison of the opsonic activity of human surfactant protein A for *Staphylococcus aureus* and *Streptococcus pneumoniae* with rabbit and human macrophages. *J Infect Dis* 1992;**167**:91-7.
116 **MacNeill IF, Porter IA, Green CA.** Staphylococcal infection in a surgical ward-a three month study. *Br Med J* 1961;**23**:798-802.
117 **Maki DG, Ringer M.** Evaluation of dressing regimens for prevention of infection with peripheral intravenous catheters: gauze, a transparent polyurethane dressing, and a iodophor-transparent dressing. *JAMA* 1987;**258**:2396-403.
118 **Martin RR, Buttram V, Besch P, Kirkland JJ, Petty GP.** Nasal and vaginal *Staphylococcus aureus* in young women: quantitative studies. *Ann Intern Med* 1982;**96(part2)**:951-3.
119 **Martin RR, White A.** The reacquisiton of staphylococci by treated carriers: A demonstration of bacterial interference. *J Lab Clin Med* 1968;**71**:791-7.

120 **Maslow JN, Mulligan ME, Arbeit RD.** Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. *Clin Infect Dis* 1993;**17**:153-64.
121 **Mest DR, Wong DH, Shimoda KJ.** Nasal colonization with methicillin resistant *Staphylococcus aureus* on admission to the surgical intensive care unit increases the risk of infection. *Anaest Anal* 1994;**78**:644-50.
122 **Mey A, Gille Y, Thivolet CH.** Carriage of *Staphylococcus aureus* and local infections in diabetic outpatients treated with insulin pen. *Diabetes Care* 1990;**13**:451-2.
123 **Miles AA, Williams REO, Clayton-Cooper B.** The carriage of *Staphylococcus (pyogenes) aureus* in man and its relation to wound infection. *J Path Bact* 1944;**56**:513-24.
124 **Millian SJ, Baldwin JN, Rheins MS, Weiser HH.** Studies on the incidence of coagulase-positive staphylococci in a normal unconfined population. *Am J Publ Health* 1960;**50**:791-798.
125 **Miller DL, McDonald JC, Jevons MP, Williams REO.** Staphylococcal diseases and nasal carriage in the Royal Air Force. *J Hyg (Camb)* 1960;**60**:451-65.
126 **Morales ED, Herwaldt L, Nettleman M.** *Staphylococcus aureus* carriage and saphenous vein harvest site infection (HSI) following coronary artery bypass surgery (CABG). *Infect Control Hosp Epidemiol* 1994;.**April**:44 (Abstract M60).
127 **Morton TM, Johnston JL, Patterson G, Archer GL.** Characterization of a conjugative staphylococcal mupirocin resistance plasmid. *Antimicrob Agents Chemother* 1995;**39**:1272-80.
128 **Mudd S.** Staphylococcal infections in the hospital and community. *JAMA* 1958;**166**:1177-8.
129 **Muder RR, Brennen C, Wagener MM, Vickers RM, Rihs JD, Hancock GA, Yee YC, Miller JM, YuVL.** Methicillin-resistant staphylococcal colonization and infection in a long-term care facility. *Ann Intern Med* 1991;**114**:107-12.
130 **Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, Korvick JA, Kauffman CA, Yu VL.** Methicillin-resistant *Staphylococcus aureus*: A consensus review of the microbiology, pathogenesis and epidemiology with implications for prevention and management. *Am J Med* 1993;**94**:313-28.
131 **Muro K, Lim BA.** A comparison of mupirocin and rifampicin in short term eradication of *Staphylococcus aureus* nasal carriage in hemodialysis patients. *J Am Soc Nephrol* 1991;**2**:340.
132 **Murray-Leisure KA, Geib S, Graceley D, Rubin-Slutsky AB, Saxena N, Muller HA and Hamory BH.** Control of epidemic methicillin-resistant *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* 1990;**11**:343-50.
133 **Musser JM.** Molecular population genetic analysis of emerged bacterial pathogens: selected insights. *Emerging Infect Dis* 1996;**2**:1-17.
134 **Nahmias AJ, Lepper MH, Hurst V, Mudd S.** Epidemiology and treatment of chronic staphylococcal infections in the household. *Am J Public Health* 1962;**52**:1828-43.
135 **Noble WC.** Carriage of *Staphylococcus aureus* and beta haemolytic streptococci in relation to race. *Acta Dermatovener* 1974;**54**:403-5.
136 **Noble WC, Rebel MH, Smith I.** An investigation of the skin flora of dialysis and transplant patients. *Br J Dermatol* 1974;**91**:201-7.
137 **Noble WC, Valkenburg HA, Wolters CHL.** Carriage of *Staphylococcus aureus* in random samples of a normal population. *J Hyg Camb* 1967;**65**:567-73.
138 **Noble WC, Willams REO, Jevons MP, Shooter RA.** Some aspects of nasal carriage of staphylococci. *J Clin Path* 1964;**17**:79-83.
139 **Noble A.** Transfer of vancomycin resistance to methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett* 1992;**93**:195-8.
140 **Ogawa SK, Yurberg ER, Hatcher VB, Levitt MA, Lowy FD.** Bacterial adherence to human endothelial cells in vitro. *Infect Immun* 1985;**50**:218-24.
141 **Olmsted SB, Norcross NL.** Effect of specific antibody on adherence of *Staphylococcus aureus* to bovine mammary epithelial cells. *Infect Immun* 1992;**60**:249-56.
142 **Oxton LL, Zimmerman SW, Roecker EB, Wakeen M.** Risk factor for peritoneal dialysis-related infections. *Perit Dial Int* 1994;**14**:137-44.
143 **Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson JS, Martone WJ.** The national nosocomial infection surveillance system. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975-1991. *Infect Cont Hosp Epidemiol* 1992;**13**:582-6.

144 **Paul MO, Lamikanra A, Aderibigbe.** Nasal carriers of coagulase-positive staphylococci in a Nigerian hospital community. *Trans Roy Soc Trop Med Hyg* 1982;**76**:319-23.
145 **Parras F, del Carmen Guerrero M, Bouza E, Jose Blazquez M, Moreno S, Cruz Menarguez M, Cercenado E.** Comparative study of mupirocin and oral co-trimoxazole plus topical fusidic acid in eradication of nasal carriage of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1995;**39**:175-9.
146 **Patti JM, Allen BL, McGavin MJ, Hook M.** MSCRAMM-mediated adherence of microorganisms to host tissue. *Annu Rev Microbiol* 1994;**48**:585-617.
147 **Peacock JE, Moorman DR, Wenzel RP, Mandell GL.** Methicillin-resistant *Staphylococcus aureus*: microbiologic characteristics, antimicrobial susceptibilities, and assesment of virulence of an epidemic strain. *J Infect Dis* 1981;**144**:575-82.
148 **Perez-Fontan M, Garcia-Falcon T, Rosales M, Rodriguez-Carmona A, Adeva M, Rodriguez-Lozano I, Moncalian J.** Treatment of *Staphylococcus* aureus nasal carriers in continuous ambulatory peritoneal dialysis with mupirocin: long-term results. *Am J Kidney Dis* 1993;**22**:708-12.
149 **Pignatari A, Pfaller M, Hollis R, Sesso R, Leme I, Herwaldt L.** *Staphylococcus aureus* colonization and infection in patients on continuous ambulatory peritoneal dialysis. *J Clin Microbiol* 1990;**28**:1898-1902.
150 **Pignatari A, Boyken LD, Herwaldt LA, Hollis R, Leme I, Sesso R, Pfaller MA.** Application of restriction endonuclease analysis of chromosomal DNA in the study of *Staphylococcus aureus* colonization in continuous ambulatory peritoneal dialysis patients. *Diagn Microbiol Infect Dis* 1992;**15**:195-9.
151 **Piraino B, Bernardini J, Sorkin M.** A five-year study on the microbiologic results of exit site infections and peritonitis in continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1987;**10**:281-6.
152 **Piraino B, Perlmutter JA, Holley JL, Bernardini J.** *Staphylococcus aureus* peritonitis is associated with *Staphylococcus aureus* nasal carriage in peritoneal dialysis patients. *Peritoneal Dial Int* 1993;**13(Suppl 2)**:5332-4.
153 **Polakov S, Richards IDG, Parker MT, Lidwell OM.** Nasal and skin carriage of *Staphylococcus aureus* by patients undergoing surgical operation. *J Hyg (London)* 1967;**65**:559-66.
154 **Poston SM, Glancey GR, Wyatt JE, Hogan T, Foster TJ.** Co-elimination of mec and *spa* genes in *Staphylococcus aureus* and the effect of *agr* and protein A production on bacterial adherence to cell monolayers. *J Med Microbiol* 1993;**39**:422-8.
155 **Public Health Laboratory Serice.** Incidence of surgical wound infections in England and Wales. *Lancet* 1960;**Sep**:659-63.
156 **Pujol M, Pena C, Pallares R, Ariza J, Ayats J, Dominguez MA, Gudiol F.** Nosocomial *Staphylococcus aureus* bacteremia among nasal carriers of methicillin-resistant and methicillin-susceptible strains. *Am J Med* 1996;**100**:509-16.
157 **Raviglione MC, Marluz P, Pablos-Mendez A, Battan R, Ottuso P, Taranta A.** High *Staphylococcus aureus* nasal carriage rate in patients with acquired immunodeficiency syndrome or AIDS-related complex. *Am J Infect Control*. 1990;**18**:64-9.
158 **Reagan DR, Doebbeling BN, Pfaller MA, Sheetz CT, Houston AK, Hollis RJ, Wenzel RP.** Elimination of coincident *Staphylococcus aureus* nasal and hand carriage with intranasal application of mupirocin calcium ointment. *Ann Intern Med* 1991;**114**:101-6.
159 **Rebel MH, van Furth R, Stevens P, Bosscher-Zonderman L, Noble WC.** The flora of renal haemodialysis shunt sites. *J Clin Path* 1975;**28**:29-32.
160 **Ridgway EJ, Wilson APR, Kelsey MC.** Preoperative screening cultures in the identification of staphylococci causing wound and valvular infections in cardiac surgery. *J Hosp Infect* 1990;**15**:55-63.
161 **Rikitoni N, Nagatake T, Sakamoto T, Matsumoto K.** The role of MRSA (methicillin-resistant *Staphylococcus aureus*) adherence and colonization in the upper respiratory tract of geriatric patients in nosocomial pulmonary infection. *Microbiol Immunol* 1994;**38**:607-14.
162 **Rountree PM.** Staphylococci harboured by people in Western highlands of New Guinea. *Lancet* 1956;**May**:719-20.
163 **Rountree PM, Loewenthal J, Tedder E, Gye R.** Staphylococcal wound infection: The use of neomycin and chlorohexidine ("Naseptin") nasal cream in its control. *Med J Aust* 1962;**2**:367-70.

164 **Saginur R, Sleigh K, Marks H, Bergeron MG, Chow AW, and the ESPRIT study group.** *Staphylococcus aureus* nasal carriage and post-operative wound infection. 8th Internatinal symposium on staphylococci and staphylococcal infections. June 23-26, Aix-les-Bains, France 1996. Abstract 308.

165 **Sanford BA, Ramsay MA.** Detection of staphylococcal membrane receptors on virus-infected cells by direct adhesin overlay. *Infect Immun* 1986;**52**:671-5.

166 **Sanford BA, Thomas VL, Ramsay MA.** Binding of staphylococci to mucus in vivo and in vitro. *Infect Immun* 1989;**57**:3735-42.

167 **Schaberg DR, Culver DH, Gaynes RP.** Major trends in the microbial etiology of nosocomial infection. *Am J Med* 1991;**91**:725-55.

168 **Schonwetter BS, Stolzenberg ED, Zasloff MA.** Epithelial antibiotics induced at sites of inflammation. *Science* 1995;**267**:1645-8.

169 **Schwab UE, Wold AE, Carson JL, Leigh MW, Cheng P, Gilligan PW, Boat TF.** Increased adherence of *Staphylococcus aureus* from cystic fibrosis lungs to airway epithelial cells. *Am Rev Resp Dis* 1993;**148**:365-9.

170 **Schwarzkopf A, Karch H.** Genetic variation in *Staphylococcus aureus* coagulase genes and limits for use as epidemiological marker. *J Clin Microbiol* 1994;**32**:2407-12.

171 **Sesso R, Draibe S, Castelo A, Sato I, Leme I, Barbosa D, Ramos O.** *Staphylococcus aureus* skin carriage and development of peritonitis in patients on continuous ambulatory peritoneal dialysis. *Clin Nephrol* 1989;**31**:264-8.

172 **Sewell CM, Clarridge J, Lacke C, Weinman EJ, Young EJ.** Staphylococcal nasal carriage and subsequent infection in peritoneal dialysis patients. *JAMA* 1982;**248**:1493-5.

173 **Sherertz RJ, Reagan DR, Hampton KD, Robertson KL, Streed SA, Hoen HM, Thomas R, Gwaltney jr JM.** A cloud adult: the *Staphylococcus aureus*-virus interaction revisited. *Ann Intern Med* 1996;**124**:539-47.

174 **Shibl AM.** Effect of antibiotics on adherence of microorganisms to epithelial cell surfaces. *Rev Infect Dis* 1985;**7**:51-65.

175 **Shinefield HR, Ribble JC, Boris M, Eichenwald HF.** Bacterial interference: its effect on nursery acquired infection with *Staphylococcus aureus*. I. Preliminary observations on artificial colonization of newborns. *Am J Dis Children* 1963;**105**:646-54.

176 **Shooter RA, Smith MA, Griffiths JD, Brown MEA, Williams REO, Rippon JE, Jevons PM.** Spread of Staphylococci in a surgical ward. *Brit Med J* 1958;**1**:607-13.

177 **Shuter J, Hatcher VB, Lowy FD.** *Staphylococcus aureus* binding to human nasal mucin. *Infect Immun* 1996;**64**:310-8.

178 **Smith JA, O'Connor JJ.** Nasal carriage of *Staphylococcus aureus* in diabetes mellitus. *Lancet* 1966;**Oct**:776-7.

179 **Smith KJ, Wagner KF, Yeager J, Skelton HG, Ledsky R.** *Staphylococcus aureus* carriage and HIV-1 disease: association with increased mucocutaneous infections as well as deep soft-tissue infections and sepsis. *Arch Dermatol* 1994;**130**:521-2.

180 **Snydman DR, Sullivan B, Gill M, Gould JA, Parkinson DR, Atkins MB.** Nosocomial sepsis associated with interleukin-2. *Ann Int Med* 1990;**112**:102-7.

181 **Soell M, Diab M, Haan-Archipoff G, Beretz A, Herbelin C, Poutrel B, Klein JP.** Capsular polysaccharide types 5 and 8 of *Staphylococcus aureus* bind specifically to human epithelial (KB) cells, endothelial cells, and monocytes and induce release of cytokines. *Infect Immun* 1995;**63**:1380-6.

182 **Steele RW.** Recurrent staphylococcal infection in families. *Arch Dermatol* 1980;**116**:189-90.

183 **Stegeman CA, Cohen Tervaert JWC, Sluiter WJ, Manson WL, de Jong PE, Kallenber CGM.** Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener's granulomatosis. *Ann Intern* Med 1994;**120**:12-7.

184 **Stokes EJ, Milne SE.** Effect of Naseptin cream prophylaxis on staphylococcal infection in adult surgical wards and infant nurseries. *J Hyg (London)* 1962;**60**:209-15.

185 **Strauss WG, Maibach HI, Shinefield HR.** Bacterial interference treatment of recurrent furunculosis. *JAMA* 1969;**208**:861-3.

186 **Stubbs E, Pegler M, Vickery A, Harbour C.** Nasal carriage of *Staphylococcus aureus* in Australian (pre-clinical and clinical) medical students. *J Hosp Infect* 1994;**27**:127-34.

187 **Sutra L, Poutrel B.** Virulence factors involved in the pathogenesis of bovine intramammary infections. *J Med Microbiol* 1994;**40**:79-89.

188 **Talon D, Rouget C, Cailleaux V, Bailly P, Thouverez M, Barale F, Michel-Briand Y.** Nasal carriage of *Staphylococcus aureus* and cross-contamination in a surgical intensive care unit: efficacy of mupirocin ointment. *J Hosp Infect* 1995;**30**:39-49.

189 **Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S, Goering R, Hancock G, Hebert GA, Hill B, Hollis R, Jarvis WR, Kreiswirth B, Eisner W, Maslow J, McDougal LK, Miller JM, Mulligan ME, Pfaller MA.** Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J Clin Microbiol* 1994;**32**:407-15.

190 **Thomas PD, Hampson FW, Hunninghake GW.** Bacterial adherence to human endothelial cells. *J Appl Physiol* 1988;**65**:1372-6.

191 **Thomas VL, Sanford BA, Ramsay MA.** Calcium- and mucin-binding proteins of staphylococci. *J Gen Microbiol* 1993;**139**:623-9.

192 **Tompkins LS, Tenover F, Arvin A.** New technology in the clinical microbiology laboratory: what you always wanted to know but were afraid to ask. *J Infect Dis* 1994;**170**:1068-74.

193 **Tuazon CU.** Skin and skin structure infections in the patient at risk: carrier status of *Staphylococcus aureus*. *Am J Med* 1984;**76(Suppl 5A)**:166-71.

194 **Tuazon CU, Perez A, Tomokauzu Kishaba, Sheagren JN.** *Staphylococcus aureus* among insulin-injecting diabetic patients. An increased carrier rate. *JAMA* 1975;**231**:1272.

195 **Tuazon CU, Sheagren JN.** Increased rate of carriage of *Staphylococcus aureus* among narcotic addicts. *J Infect Dis* 1974;**129**:725-7.

196 **Valeriano-Marcet J and Spiera H.** Treatment of Wegener granulomatosis with sulfa-methoxazole-trimethoprim. *Arch Intern Med* 1991;**151**:1649-52.

197 **Van Belkum A, Kluytmans J, van Leeuwen W, Bax W, Quint W, Peters E, Fluit A, Vandenbroucke-Grauls C, van den Brule A, Koeleman H, Melchers W, Meis J, Elaichouni A, Vaneechoute M, Moonens F, Maes N, Struelens M, Tenover F and Verbrugh H.** Multicenter evaluation of arbitrarily primed PCR for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1995;**33**:1537-47.

198 **Van Belkum A, Riewerts Eriksen NH, Sijmons M, van Leeuwen W, VandenBergh M, Kluytmans J, Espersen F, Verbrugh HA.** Nasal colonisation by *Staphylococcus aureus*: do coagulase and protein A polymorphisms contribute to persistency? *J Med Microbiol* In press

199 **Van Belkum A, van leeuwen W, Kluytmans J, Verbrugh H.** Moleculair nosocomial epidemiology: High speed typing of microbial pathogens by arbitrary primed PCR assays. *Infect Control Hosp Epidemiol* 1995;**16**:658-66.

200 **Van Belkum A.** DNA fingerprinting of medically important microorganisms by use of PCR. *Clin Microbiol Rev* 1994;**7**:174-84.

201 **Vann JM, Hamill RJ, Albrecht RM, Mosher DF, Proctor RA.** Immunoelectron microscopic localization of fibronectin in adherence of *Staphylococcus aureus* to cultured bovine endothelial cells. *J Infect Dis* 1989;**160**:538-42.

202 **Vaudaux P and Waldvogel FA.** Methicillin resistant strain of *Staphylococcus aureus*: Relationship between expression of resistance and phagocytosis by polymorphonuclear leucocytes. *J Infect Dis* 1979;**7**:223-8.

203 **Vercelotti GM, Lussenhop D, Peterson PK, Furcht LT, McCarthy JB, Jacob HS, Moldow CF.** Bacterial adherence to fibronectin and endothelial cells: A possible mechanism for bacterial tissue tropism. *J Lab Clin Med* 1984;**103**:34-43.

204 **Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I.** Methicillin-resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect Dis* 1994;**13**:50-5.

205 **Voss A, Doebbeling BN.** The worldwide prevalence of methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrobial Agents* 1995;**5**:101-6.

206 **Ward TT.** Comparison of in vitro adherence of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* to human nasal epithelial cells. *J Infect Dis* 1992;**166**:400-4.

207 **Weinke T, Schiller R, Fehrenbach FJ, Pohle HD.** Association between *Staphylococcus aureus* nasopharyngeal colonization and septicemia in patients infected with the human immunodeficiency virus. *Eur J Clin Microbiol Infect Dis* 1992;**11**:985-9.

208 **Weinstein HJ.** The relation between nasal-staphylococcal-carrier state and the incidence of postoperative complications. *New Engl J Med* 1959;**260**:1303-8.
209 **White A.** Increased infection rates in heavy nasal carriers of coagulase-positive staphylococci. *Antimicrob Agents Chemother* 1963;**30**:667-70.
210 **Williams REO.** Healthy carriage of *Staphylococcus aureus*: Its prevalence and importance. *Bacteriol Rev* 1963;**27**:56-71.
211 **Williams REO, Blowers R, Garrod LP, Shooter RA.** Hospital infection, causes and prevention. 1966, 2nd edition. Lloyd-Luke (medical books) Ltd.
212 **Williams REO, Patricia-Jevons M, Shooter RA, Hunter CJW, Girling JA, Griffiths JD, Taylor GW.** Nasal staphylococci and sepsis in hospital patients. *Br Med J* 1959;**Oct**:658-62.
213 **Winkler J, Block C, Leibovici L, Faktor J, Pitlik SD.** Nasal carriage of *Staphylococcus aureus*: correlation with hormonal status in women. *J Infect Dis* 1990;**162**:1400-2.
214 **Witt DJ, Craven DE, McCabe WR.** Bacterial infections in adult patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *Am J Med* 1987;**82**:900-6.
215 **Working party report.** Revised guidelines for the control of epidemic methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 1990;**16**:351-77.
216 **Wright AE.** Wound infections and some new methods for the study on the various factors which come into consideration in their treatment. 1915, University of London Press Ltd.
217 **Yu VL, Goetz A, Wagener M, Smith PB, Rihs JD, Hanchett J, Zuravleff JJ.** *Staphylococcus aureus* nasal carriage and infection in patients on hemodialysis. *N Engl J Med* 1986;**315**:91-6.
218 **Yzerman EPF, Boelens HAM, Tjhie JHT, Kluytmans JAJW, Mouton JW, Verbrugh HA.** ΔAPACHE II for predicting course and outcome of nosocomial *Staphylococcus aureus* bacteremia and its relation to host defense. *J Infect Dis* 1996;**173**:914-9.
219 **Zimmerman SW, Ahrens E, Johnson CA, Craig W, Leggett J, O'Brien M, Oxton L, Roecker EB, Engeseth S.** Randomized controlled trial of prophylactic rifampicin for peritoneal dialysis-related infections. *Am J Kidney Dis* 1991;**18**:225-31.
220 **Zimmerman SW, O'Brien M, Wiedenhoeft FA, Johnson CA.** *Staphylococcus aureus* peritoneal catheter related infections: A cause of catheter loss and peritonitis. *Periton Dialysis Int* 1988;**8**:191-4.

CHAPTER FOUR
MOLECUR BIOLOGICAL TYPING METHODS

PART ONE
MULTICENTER EVALUATION OF ARBITARILY PRIMED PCR FOR TYPING OF *STAPHYLOCOCCUS AUREUS* STRAINS

Alex van Belkum, Jan Kluytmans, Willem van Leeuwen, Rene Bax, Wim Quint, Edith Peters, Ad Fluit, Christina Vandenbroucke-Grauls, Adriaan van den Brule, Hans Koeleman, Willem Melchers, Jacques Meis, Abdeslam Elaichouni, Mario Vaneechoutte, Francoise Moonens, Nicole Maes, Marc Struelens, Fred Tenover and Henri Verbrugh

Adapted from: *J Clin Microbiol* 1995;**33**:1537-47

SUMMARY

Fifty-nine isolates of *Staphylococcus aureus* and a single strain of *Staphylococcus intermedius* were typed by arbitrary primed PCR (AP-PCR). To study reproducibility and discriminatory abilities, AP-PCR was carried out in seven laboratories with a standardized amplification protocol, template DNA isolated in a single institution, and a common set of three primers with different resolving power. The 60 strains could be divided into 16 to 30 different genetic types, depending on the laboratory. This difference in resolution was due to differences in technical procedures (as shown by the deliberate introduction of experimental variables) and/or the interpretation of the DNA fingerprints. However, this did not hamper the epidemiologically correct clustering of related strains. The average number of different genotypes identified exceeded those of the more traditional typing strategies.[22] Comparison of AP-PCR with pulsed-field gel electrophoresis (PFGE) indicated the existence of strains with constant PFGE but variable AP-PCR types. The reverse (constant AP-PCR and variable PFGE patterns) was also observed. This indicates additional resolution for combined analyses. It is concluded that AP-PCR is well suited for genetic analysis and monitoring of nosocomial spreading of staphylococci. The interlaboratory reproducibility of DNA-banding patterns and intralaboratory standardization need improvement.

INTRODUCTION

Numerous procedures for comparison of bacterial isolates have been developed.[2,11,13,15] These procedures are important in investigations of strain origin, clonal relatedness among strains, and epidemiology. For *Staphylococcus aureus*, it has been demonstrated that most of the typing procedures can be applied successfully to obtain epidemiologically useful data. Tenover *et al.*[22] compared 12 typing strategies and concluded that DNA-based typing methods and immunoblotting are best suited for epidemiological analyses. With the exception of biotyping, which appeared to produce too many subtypes, no single technique proved overtly superior or inferior. When all procedures were compared, unrelated strains were grouped with differing frequencies. This comparative analysis of typing procedures provides a reference scheme for rating novel typing strategies against the more established methods. Recently, a large number of reports describing the use of the PCR for genetic typing of medically important microorganisms have appeared.[23,26] By arbitrarily amplifying variable regions in the bacterial genome (arbitrarily primed PCR [AP-PCR]), isolate-specific DNA fingerprints can be obtained in a rapid and reproducible manner. In most cases, these analyses are not accompanied by detailed comparisons with the results of alternative typing procedures. For *S. aureus*, for instance, several studies have compared AP-PCR with only a single other technique.[20,21,25,26]

The present study was undertaken to determine the reproducibility and discriminatory abilities of AP-PCR when compared with other staphylococcal typing procedures. To this end, the *S. aureus* strains that were studied previously by Tenover *et al.*[22] were typed in multiple AP-PCR assays with three different arbitrary primers, guided by a standard amplification protocol and performed independently in seven different laboratories.

MATERIALS AND METHODS

Bacterial strains and description of isolates. Fifty-nine isolates of *S. aureus* were included in this study. All isolates were identified and confirmed to be *S. aureus* by standard biochemical methods.[12] The strains have been described before,[22] and 40 of them were derived from five well-documented outbreaks. The 19 additional isolates are epidemiologically unlinked. A single isolate of *S. intermedius* was included.

The 60 strains were divided into three groups (SA, SB and SC), some of whose key features are summarised below (Table 1). Group SA contains the strains involved in outbreaks that occurred in two nursing homes (strains labeled NH1 and NH2). Strain SA-04 is ATCC 12600 (American Type Culture Collection, Rockville, Md.). This set also contains a number of independent isolates from the Centers for Disease Control and Prevention, the *S. intermedius* strain, and three isolates of phage type 47/54/75/77/83A. These latter strains were isolated in three different American states during three different years.

Group SB contains strains from outbreaks I and II, eight unrelated strains, and, again, *S. aureus* ATCC 12600 (SB-07). Strains from outbreak I are methicillin resistant and were isolated in the Iowa Veterans Affairs Medical Center (18). Outbreak II was related to a contaminated anaesthetic.[6]

Group SC contains strains from outbreaks III and IV, an unrelated control, and ATCC 12600 (SC-03). Outbreak IV was again anaesthetic related,[6] although it differed from outbreak II. Outbreak III was caused by 10 methicillin-resistant strains in the Sepulveda Veterans Affairs Medical Center, Sepulveda, California.[9]

Bacterial typing studies. All isolates were typed previously by a number of procedures.[22] Antibiograms and biotypes were determined, and bacteriophage sensitivity was assayed. Restriction fragment length polymorphisms (RFLP) were screened using enzymatic digestion

TABLE 1: *general survey of phenotypic and genotypic typing data in comparison with the results from the multicenter study on ap-pcr mediated dna fingerprinting for 60 Staphylococcus strains*

Strain[a]	Out-break[b]	Ox[ec]	Phage type	Anti bio gram	Bio type	AP-PCR datasets[d]					
						Ia	Ib	II	III	IV	V
SA-16	NO	S	NR	I	INT[i]	HEH	FEF	HEG	GDH	FDF	FDG
SA-04	NO	S	6/47/54/75	B	A2B	BBB	BBB	BBB	BBB	BBB	BBC
SA-12	NO	R	47/54/75/77/83A	G	A3B	AAA	AAA	AAA	AAA	AAA	AAA
SA-18	NO	R	47/54/75/77/83A	J	A3B	AAA	AAA	AAA	AAA	AAA	AAA
SA-20	NO	R	47/54/75/77/83A	K	A3B	AAA	AAA	AAA	AAA	AAA	AAA
SA-06	NO	I	NR	C	A3B	CAC	AAC	CAC	CAC	AAA	AAB
SA-07	NO	S	53/+	D	H4	DAD	AAC	DAC	AAD	CAA	AAD
SA-08	NO	R	54/75/77/81	E	I2B	ECE	CCD	ECD	DBE	DBC	CBE
SA-11	NO	R	NR	F	A2B	ECF	CCD	ECE	DBF	DBD	CBF
SA-01	NH1	R	54/77	A1	A1B	AAA	AAA	AAA	AAA	AAA	AAA
SA-09	NH1	R	54/77	A	A1B	AAA	AAA	AAA	AAA	AAA	AAD
SA-03	NH1	R	47/54/75/77	A2	A3B	AAA	AAA	AAA	AAA	AAA	AAB
SA-13	NH1	R	54/77	A3	A1B	AAG	AAA	AAA	AAA	AAA	AAA
SA-14	NH1	S	54/75/77	H	B1B	GDG	EDE	GDF	FCG	-CE	ECA
SA-19	NH1	R	54/77	A4	G1B	AAA	AAA	AAA	AAA	AAA	AAB
SA-17	NH2	R	54/75/77	A	C3B	AAA	AAA	AAA	AAA	AAA	AAB
SA-02	NH2	R	75/77	A	A3B	AAA	AAA	AAA	AAA	AAA	AAA
SA-15	NH2	R	77	A	A3B	AAA	AAA	AAA	AAA	AAA	AAA
SA-05	NH2	R	77	A	A3B	AAA	AAA	AAA	AAA	AAA	AAB
SA-10	NH2	R	77	A	A3B	FAA	DAA	FAA	EAA	EAA	DAA
SB-07	NO	S	6/47/54/75	C	A2B	BBB	BBB	BBB	JBB	BBH	-BC
SB-03	I	R	75/+	A	C4	AAA	AAA	AFA	HEA	AE-	AEA
SB-05	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEA	AEA
SB-10	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEA	AEK
SB-12	I	R	75/+	A	C4	AAA	AAA	AFA	HEA	AEL	AEK
SB-15	I	R	75/77/83	A	C4	AAA	AAA	AFA	HEA	AEL	AEA
SB-19	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEL	AEA
SB-20	I	R	75/+	A	A4	AAA	AAA	AFA	HEA	AEL	AEK
SB-01	NO	R	75/77	A	A4	AAA	AAA	AFA	HEA	AEA	AEA
SB-16	NO	R	75/77/83A	A	A4	AAA	AAA	AFA	HEA	AEL	AEA
SB-18	NO	R	75/+	A	C4	AAA	AAA	AFA	HEA	AEL	AEA
SB-17	NO	I	96	E	B3B	LDL	JIM	NJM	OCQ	LCO	KCM
SB-14	NO	R	47/54/75/77/83A	A1	A3B	AAG	AHL	AAL	HAP	AAN	AAA
SB-08	NO	S	95	B1	C4	JGJ	HBI	JHI	KGM	IGI	IGI
SB-02	II	S	3A/55	B	B1B	IFI	GFH	IGH	IFK	GFG	HFH
SB-04	II	S	3A/55	B	D1B	IFI	GFH	IGH	IFL	HFG	-FH
SB-06	II	S	3A/55	B	B1B	IFI	GFH	IGH	IFK	HFG	HFH
SB-11	II	S	3A/55	B	B3B	FFK	GFK	LGK	MFO	KHK	JFL
SB-09	NO	S	3A	D	D3B	BFI	IDJ	KIJ	LBN	JBJ	BBJ
SB-13	NO	S	3A	B2	D3B	KFI	GGJ	MIJ	NBN	RBM	BBJ
SC-03	NO	S	6/47/54/75	C	A2B	BBB	BJB	BBB	JBB	OBP	LBC
SC-01	III	R	75	A	A1B	MFB	BJB	OBB	PBB	MBP	LBB
SC-04	III	R	75	A	A1B	MFB	BJB	OBB	PBB	MBP	LBB
SC-05	III	R	NR	A1	A1B	MFB	BJB	OBB	PBB	-BP	LBB
SC-09	III	R	75	A	A1B	MFB	BJB	OBB	PBB	MBP	LBN
SC-11	III	R	75	E	A1B	MFB	BJB	OBB	PBB	MBP	LBN
SC-12	III	R	75	A2	A1B	MFB	BJB	OBB	PBB	MBP	LBN
SC-14	III	R	75	A2	B2B	MFB	BJB	OBB	PBB	MBP	LBN
SC-15	III	R	75	A	A1B	MFB	BJB	OBB	P-B	MBP	LBN
SC-17	III	R	75	A	A1B	MFB	BJB	OBB	P-B	MBP	LBN
SC-20	III	R	75	A	A1B	MFB	BJB	OBB	PB-	MBP	LBN
SC-08	NO	S	NR	B	B3A	JHD	HKI	JHI	KGM	PII	MGI
SC-02	IV	S	52/52A/80/47/54 83A/84/95	B	E1B	NGJ	HKI	JHI	KGM	NII	MGI
SC-06	IV	S	95	B	J1B	JHD	HKI	JHI	KGM	PJI	MGI
SC-07	IV	S	95	D	I1A	JHD	HKI	JHI	KGM	PJI	MGI
SC-10	IV	S	52A/79/80/47/54 75/77/83A/95	B	I2A	JHD	HKI	JHI	KGM	-II	MGI
SC-13	IV	S	95	B1	I1B	JHD	HKI	JHI	KGM	NII	MGI
SC-16	IV	S	95	B1	I1B	JHD	HKI	JHI	KGM	PJI	MGI
SC-18	IV	S	95	F	I3B	JHD	HKI	JHI	KGM	PJI	MGI
SC-19	IV	S	95	B1	D1A	JHD	HKI	JHI	KGM	PII	MGI
No. of types[j]		3	19	11	15	20	16	18	21	30	21

a The strain numbers that are underlined are three isolates of a single ATCC strain

b NO, not in epidemiologically related cluster; I to IV, outbreak number; NH1/2, nursing home pseudo outbreak.

c Ox[s], oxacillin susceptibility test result.

d Columns numbered Ia through VII give surveys of the AP-PCR data as determined in the different institutions. The three letter code summarises the typing results per primer used (first digit, primer 1; second digit, primer 7; third digit, primer E2). Data represented by a capital letter given in a certain column may be different from the same character in another column. Underlining in the in the AP-PCR datasets indicates minor differences in DNA staining intensities.

VI	VII	Plasmid[e]	Ribotype[f]	PFGE	FIGE	Immunoblot	MLEE	Is[g]	Coagulase PCR[h]	RFLP type
GFG	DDC	NP	de	I	VII	K	F	NH	0.0	NH:NH:NH:NH
AAA	BBA2	B	fi	E	IV	D	E	NH	2.1	NH:X:4:NH
AAA	AAA1	NP	bb	J	IC2	A	A5	C	9.0	I:A:1:NH
ABA	AAA1	I	bb	J	IC3	A2	A3	C	9.0	I:A:1:NH
ABB	EAA1	J	bb	J	IC1	A1	A1	C	9.0	I:A:1:NH
ABB	AAA1	C	aa	C	III	A4	A4	B	9.0	II:NH:1:a
CBA	AAA1	D	bc	B	V	C	A2	NH	9.0	NH:NH:1:NH
DDD	CB'B	E	ed	G	IIA	E1	D1	D	7.0	I:NH:6:NH
DDE	CB'B	E	gd	F	IIB	E2	D2	G	7.0	II:NH:6:NH
AAA	AAA1	A	aa1	K1	IB	A1	A1	A	9.0	I:A:5:a
ABA	AAA1	NP	aa1	K2	IB	A1	A1	A	9.0	I:A:5:a
ABB	AAA1	NP	aa	A	IA	A	A1	C	9.0	I:A:1:NH
AAA	AAA1	G	aa	A	IA	A3	A2	A	9.0	I:A:1:a
FEF	ACA3	H	ci	H	VI	E3	C	NH	9.0	NH:NH:1:NH
ABB	EAA1	A	aa1	K3	IB	A1	A1	A	9.0	I:A:1:a
ABB	EAA1	A	aa	A	IA	A	A1	A	9.0	I:A:1:a
AAA	AAA1	A	aa	A	IA	A	A1	A1	9.0	I:A:1:b
AAB	AAA1	A	aa	A	IA	A1	A5	A1	9.0	I:A:1:a
ABB	AAA1	A	aa	A	IA	A	A1	A	9.0	I:A:1:a
EAA	JAA1	A	aa	D	ID	A1	B	A	9.0	I:A:1:a
BCC	BBA1	D	ci	D	IIB3	D'	B3	NH	2.1	NH:X:4:NH
HGA	EEA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
HGA	EEA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
HGA	HEA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
HGA	AAA1	C	aa	A1	IA	A6	A1	E	9.0	I:A:1:a
HGA	AAA1	C	aa	A	IA	A6	A1	E	9.0	I:A:1:a
HGA	AAA1	C	aa	A	IA	A5	A1	E	9.0	I:A:1:a
HGA	AAA1	C	aa	A	IA	A5	A1	E	9.0	I:A:1:a
HGA	EEA1	A	aa	A1	IB1	A5	A1	E	9.0	I:Y:1:a
HGA	AAA1	A	aa	A1	IB1	A5	A1	E	9.0	I:Y:1:a
HGA	AAA1	J	aa	A	IA	A7	A1	E1	9.0	I:A:1:a
NKM	ACA6	I	fj	E	IV	G	A2	NH	6.0	NH:NH:1:NH
AAF	AAA3	H	ea	A2	IB2	A5	A3	D	9.0	I:A:1:NH
JII	GFG	E	dd1	F	III	E5	C	NH	2.0	NH:NH:1:NH
IHH	FBD	B	bb	B	IIA	D1	B1	NH	6.0	NH:NH:7:NH
IHH	FBD	B	bb	B	IIA	D1	B1	NH	6.0	NH:NH:7:NH
IHH	FBD	B	bb	B	IIA	D1	B1	NH	6.0	NH:NH:7:NH
LHK	ABE	G	b1b	C	IIB2	D2	B1	NH	14.0	NH:NH:7:NH
KJJ	HBE	F	bb	B	IIA	D1	B1	NH	6.0	NH:Z:7:NH
MIL	ABF	G	bb	B1	IIB1	E6	B2	NH	6.0	NH:NH:7:NH
BCP	ABA4	C	ai	C	III	D	B	NH	2.1	NH:NH:4:NH
OLN	ABA4	A	ab	A	IA	F	A1	F	10.0	I:A:4:a
OLN	ABA4	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
OLN	ABA4	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
OCN	IBA5	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
OCN	IBA5	NP	ab	A	IB	F	A1	NH	10.0	I:A:4:NH
OLN	IBA5	A	ab	A	IA	F	A1	F	10.0	I:A:4:a
OLN	IBA5	A	ab	A	IA	F	A2	F	10.0	I:A:4:a
OCN	IBA5	D	b2b	A	IA	F	A1	F	10.0	I:A:4:a
JIN	IBA5	A	ab	A	IA	F	A1	F	10.0	I:A:4:a
JIN	IBA5	D	ab	A	IA	F	A1	F	10.0	I:A:4:a
JIH	GFG	E	b1g	B1	II	E7	A3	NH	2.0	NH:NH:1:NH
JIO	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
JIH	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
JIQ	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
JIH	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
JII	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
OLI	GFG	B	ag	B	II	H	D1	NH	2.0	NH:NH:1:NH
OLI	GFG	B	bg	B	II	E7	C1	NH	2.0	NH:NH:1:NH
JOI	GFG	B	bg	B	II	E7	D2	NH	2.0	NH:NH:1:NH
28	19	10	15	11	7	7	6	8	8	17

e Plasmid, plasmid restriction profile; NP, no plasmids.
f Ribotyping results obtained with *Hind*III and *Cla*I, respectively.
g IS, insertion sequence; NH, no hybridisation.
h Coag PCR, coagulase gene PCR typing.
i INT, *S. intermedius* biotype.
j In the cumulative number of types, subnumbers are counted as a single type number.

of plasmid DNA, variable gene probes, or DNA probes derived from insertion elements (IS mapping). DNA macrorestriction fragments were separated by field inversion gel electrophoresis (FIGE) and pulsed-field gel electrophoresis (PFGE). Multilocus enzyme electrophoresis (MLEE) and immunoblotting were also performed, as were ribotyping and restriction enzyme analysis of PCR fragments derived from the staphylococcal coagulase gene.

PCR Multicenter study design. Participants were from seven institutions: two Belgian institutes (Hopital Erasme, Brussels; and University Hospital of Ghent, Ghent), and five Dutch hospitals (University Hospital Radboud, Nijmegen; University Hospital Utrecht, Utrecht; Diagnostic Centre SSDZ, Delft; Free University Hospital, Amsterdam; and University Hospital Dijkzigt, Rotterdam). The study was coordinated at the Dijkzigt Hospital, where the AP-PCR assays were performed in duplicate by two individuals following slightly different experimental protocols. All participants had experience in performing AP-PCR. This guarantees intralaboratory reproducibility of the assays. For this reason, the participants were also allowed to process the *S. aureus* DNA samples according to their own, optimised AP-PCR protocol. Results are presented anonymously, and datasets are numbered from I through VII (sets Ia and Ib derive from the coordinating laboratory).

To prevent interlaboratory variation due to different DNA extraction protocols, bacterial DNA, and not the organisms, was distributed from the coordinating center to the participating laboratories. Primers were aliquoted in Rotterdam as well and shipped together with the DNA preparations. In this way, the variables of bacterial cultivation, DNA isolation, and primer quality were controlled. This implies, however, that the results obtained during this study may differ from those that would have been obtained if bacterial strains, rather than DNA, had been distributed. DNA amplification was performed in the different laboratories with the locally available equipment and PCR ingredients. Gels containing the amplified DNA were photographed, and the results were interpreted locally according to the researchers' individual standards. Generally, differences in the number of bands indicated a novel type. Variations in band-staining intensities were disregarded. Interpretation was performed without knowledge of epidemiological relatedness. The fingerprint types were transformed in a cumulative three-letter code (one letter per type per primer) and sent to Rotterdam, where a comparative analysis was carried out. Results were studied with respect to reproducibility of the DNA fingerprints (and the accompanying interpretation and strain grouping), relation to the results obtained by other typing procedures, and epidemiological value. When possible, data were further analyzed with Gelcompar Software (Applied Maths, Kortrijk, Belgium). Pictures were digitized with a Hewlett-Packard HP Scanjet IIc document scanner. After conversion and visual normalisation, the data were analyzed. Degrees of homology were determined by Dice comparisons, and clustering correlation coefficients were calculated by the unweighted pair group method with arithmetic averages (UPGMA).

AP-PCR. A description of the three PCR-related procedures is given below. This protocol served as a reference manual. Specific deviations from this protocol are summarised per institute in Table 2.

(i) Cultivation of bacteria and isolation of DNA. Bacteria were grown in suspension in brain heart infusion (BHI) broth for 18 h at 37°C. Approximately 100 μl of a bacterial pellet was suspended in 150 μl of 25 mM Tris • HCl (pH 8.0)-50 mM glucose-10 mM EDTA. Lysostaphin (75 μl of a 100-μg/ml solution) was added, centrifuged, and the mixture was incubated at 37°C for 1 h. Spheroplasts were lysed by the addition of 1 ml of 4 M guanidinium isothiocyanate-50 mM Tris • HCl (pH 6.4)-3 mM EDTA-1% (wt/wt) Triton-X100. To immobilize and purify the DNA, 50 μl of a Celite suspension (0.2 g/ml; Janssen Pharmaceuticals, Beerse, Belgium) was added. The entire mixture was shaken for 15 s and incubated at room temperature for 10 min. After centrifugation, the supernatant was discarded; the pellet was

washed once with 1 ml of lysis buffer, twice with lysis buffer without EDTA and Triton X-100; twice with 70% ethanol in water, and, finally, once with acetone. The Celite pellet was dried in vacuo. Between 100 and 400μl of 10 mM Tris • HCl (pH 8.0)-1 mM EDTA was added, and DNA was eluted by incubation at 56°C for 10 min, interrupted by short periods of vortexing. The supernatant containing the DNA was separated from the Celite by centrifugation. The DNA concentration was determined by spectrophotometry at 260 nm, and DNA was stored at -20°C. Stock solutions of bacterial DNA were adjusted to a concentration of 5 ng/μl.

(ii)PCR. When *Tth* polymerase (SuperTaq; HT Biotechnology, Cambridge, United Kingdom) was used, the amplification conditions described below guaranteed optimal performance for this particular enzyme. The use of other enzymes usually required modification of the buffer conditions used during PCR (Table 2) and may lead to different AP-PCR results. PCR was performed with a buffer system containing 10 mM Tris • HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X100, 0.2 mM deoxynucleoside triphosphates (dNTPs), 50 pmoles of primer and 0.2 U of the *Tth* polymerase, to which DNA was added (50 ng per amplification). The PCR mixtures were overlaid with 100 μl of mineral oil. Cycling was performed in Biomed PCR machines (Model 60) and consisted of the following steps: predenaturation at 94°C for 4 min followed by 35 cycles of 1 min at 94°C, 1 min at 25°C, and 2 min at 74°C. Amplified DNA was stored at -20°C. The primers used to discriminate *S. aureus* strains were RAPD1 (GGTTGGGTGAGAATTGCACG), RAPD7 (GTGGATGCGA) and ERIC2 (AAGTAAGTGACTGGGGTGAGCG).[25,26,28]

(iii) Electrophoresis. Amplification products were separated by electrophoresis in 5 mm thick 1.5% agarose gels (Hispanagar; Sphaero Q, Leiden, The Netherlands). Gels were run in 0.5μ Tris-borate-EDTA (TBE) at a constant current of 100 mA for 2 h. Prior to electrophoresis, samples were mixed with a fivefold concentrated layer mix consisting of 50% glycerol in water and 0.8 mg bromophenol blue per ml. Then 35 μl of the amplified material was loaded on the gel, and a molecular weight marker was run in parallel with the AP-PCR samples. Gels were stained after electrophoresis by addition of 10 μl ethidiumbromide (10 mg/ml) to a total volume of 300 ml of 0.5μ TBE. The gels were photographed with a Polaroid MP4 Landcamera and Polaroid 57 High Speed films, with an exposure time of 0.125 to 0.25 sec (diaphragm F5.6). Table 2 surveys the differences among the electrophoresis conditions as applied in the different laboratories.

RESULTS

PCR Fingerprinting. An overview of the typing results is given in Table 1. The AP-PCR data are displayed in separate columns, one per participating research center, except for the coordinating laboratory, where the assays were performed in duplicate (Ia and Ib). Figure 1 gives an example of a complete set of gel pictures obtained for the 60 strains with the three AP-PCR primers. Table 3 displays the number of genotypes that were detected in the participants' laboratories. When primer 1 was used, the overall number of types varied from 10 to 17, with a mean of 14 types. The mean numbers for primers 7 and E2 were 9 and 14, respectively. With this set of strains, the discriminatory power of primers 1 and E2 is over 60% higher than that of primer 7.

The overall number of DNA bands generated per primer does not correspond to the number of detectable genotypes. Dataset IV, displaying 30 different genocodes (Tables 1 and 3) was produced from relatively small numbers of DNA fragments synthesised: seven, five, and seven fragments for primers 1, 7, and E2, respectively. These are smaller numbers than those found by the group describing the smallest number of genotypes (dataset Ib, with 16 types deduced from fingerprints consisting of 9, 9, or 11 bands for individual fingerprints). The maximum number of bands was observed when primer 1 was used.

Table 2: *Survey of experimental variables with respect to PCR fingerprinting performed in the different participating laboratories*

	Value for laboratory							
Variable	Ia	Ib	II	III	IV	V	VI	VII
Incubation volume (µl)	100	100	100	50	100	100	50	50
Polymerase type*	*Tth*	*Tth*	*Tth*	*Tth*	*Taq*	*Thp*	*Taq*	*Taq*
Amt of polymerase (u)	0.20	0.20	0.25	0.10	1.25	0.25	1.00	0.25
Mg^{2+} conc (mM)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
dNTP conc (µM)	40	40	40	100	200	40	200	200
KCl conc (mM)	50	50	50	50	50	_	50	50
PCR machine	Biomed	Biomed	Biomed	Hybaid	Biomed	P.E.	P.E.	P.E.
Sample size (%)†	30	30	30	50	25	30	30	30
Polaroid‡	57/3,000	57/3,000	52/400	665/80	665/80	667/3,000	667/3,000	665/80
Type of agarose§	Pron.	Pron.	Pron.	MP	Pron.	MP	UP	MP
% agarose	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Voltage	150	150	200	100	100	100	130	150
Migration (cm)	17 cm	10 cm	10 cm	8 cm	10 cm	10 cm	8-9 cm	8-10 cm
Ethidium bromide¥	-	-	+	+	+	+	-	+

* *Thp* (Thermoperfect DNA polymerase, Integro, Zaandam, The Netherlands); *Tth* (Supertaq, Sphaero Q, Leiden, The Netherlands); *Taq* (Taq polymerase, Cetus, Emeryville, Calif.).
* in this case no KCl was present, instead, 50 mM $(NH_4)_2SO_4$ was included.
† The sample size indicates the amount of the amplification reaction that has been separated electrophoretically.
‡ The description of the photographs includes the type and the sensitivity in ASA values.
§ Pron. (Pronarose, Hispanagar, Burgos, Spain); MP (multi purpose agarose Boehringer Mannheim); UP (Ultra Pure Agarose, Gibco/BRL, Breda, The Netherlands).
¥ The presence (+) or absence (-) of ethidium bromide during the electrophoresis is indicated.

Table 3: *Number of genetic variants detected with the individual PCR primers as independently documented by the seven participating laboratories.*

Participating center	No. detected with primer			Overall no.	No. of unique strains
	1	7	E2		
Ia	14	8	11	20	13
Ib	10	11	11	16	9
II	15	10	13	18	12
III	16	7	17	21	16
IV	17	10	16	30	15
V	13	7	14	21	11
VI	15	12	17	28	15
VII	10	7	12	19	11
mean	14	9	14	22	13

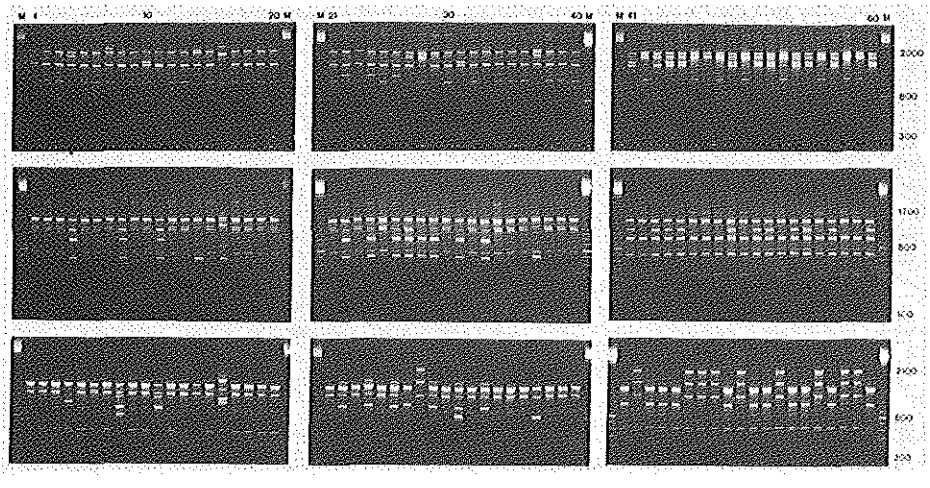

Figure 1 *AP-PCR for the 60 staphylococcal strains: survey of the experimental results belonging to dataset Ia. From left to right the strains are numbered according to Table 1, from top to bottom the primer species (primer 1, 7 and E2) has been varied. The outer lanes in all nine panels contain molecular length markers, the size of which is indicated on the right. The interpretation of the banding patterns is given in Table 1 in the column identified as Ia.*

The mean score for this primer, averaged among the groups, is 11.4 DNA fragments. For primers 7 and E2, these numbers are 6.6 and 9.6 fragments, respectively. There is no apparent variation in the average length of the fragments, as demonstrated by a survey of the cumulative results obtained by application of primer 1 (Figure 2). Although most of the types were found in all laboratories, some additional bands gave rise to additional genotypes. Discordant results can be observed. The data obtained with primer E2 seemed to be the most variable (results not shown). When the overall number of combined genocodes is considered, major differences among laboratories are encountered. The number of types varies between 16 and 30, with a mean of 22 types identified.

When the lengths of the DNA fragments generated by the individual PCR tests were investigated, primer 1 was found to generate amplicons with an overall length of approximately 11,000 bp. For primers 7 and E2, these values are 4,000 and 5,500 bp, respectively. These differences are not reflected in the overall number of detectable PCR types (which is 14 for both primer 1 and E2). Primer 7, which detected the smallest number of types, is also associated with the shortest cumulative length of the DNA fragments synthesised. There is an apparent variation in the number and size of fragments generated per primer species. For primer 1, this number varies from 6.4 to 11.4 on average. The numbers for primers 7 and E2 are 3.7 to 6.6 and 6.0 to 9.6, respectively. In general, fragments vary in length from 0.15 to over 2 kbp.

Epidemiological considerations. Analysis of the strains from outbreak IV illustrates that the data obtained by five of seven laboratories group these isolates into a homogeneous genotype that is not encountered in the rest of the collection, with the exception of a single strain (SC-08). These data are similar to those obtained by oxacillin susceptibility testing, plasmid typing, ribotyping, PFGE, FIGE, immunoblotting, IS mapping, PCR typing and RFLP mapping.[22] Two of the seven laboratories detected three to six different types in this group of eight bacterial isolates. The results collected for the strains from outbreak III are similar. In this case, four of seven datasets demonstrated the homogeneity of this subgroup. Three participants identified two or three different types. Interestingly, in two of these laboratories, where the same subtypes are established, the differences were limited to data obtained by only one of the PCR primers. Again, the PCR data are in general agreement with those obtained by the other typing techniques. The four strains from outbreak II are split into two types: three are identical (five of seven laboratories) or very similar (two of seven laboratories), whereas a single strain (SB-11) appears to be different. The other datasets confirm this observation. Results with strains from outbreak I and the nursing home (NH1 and NH2) conform to those of the other typing procedures. Since 19 non-outbreak-related strains are included in the collection, this implies that the resolution of PCR fingerprinting varies from approximately 50% to nearly 90%, since between 9 and 16 unique types were identified depending on the institution. It is assumed that all 19 non-outbreak-related strains are indeed genetically independent.

Typeability and reproducibility. All strains were typeable by PCR. Four of seven labs obtained 100% typeability; negative results seen by other participants were due to technical inadequacy not related to bacterial genome structure. The overall mean level of typeability was 99.5%. This makes PCR-mediated typing preferable in principle over phage typing, plasmid typing, some of the RFLP approaches, and IS mapping, which all leave an appreciable percentage of strains untypable.

Discriminatory power. PCR fingerprinting was not able to detect differences between strain SA-12, SA-18, and SA-20. These strains are also identical by phage typing, ribotyping, PFGE, and IS mapping. Since these strains were derived from diverse origins, it seems likely that certain clones of *S. aureus* spread easily and remain genotypically constant. On the other hand, several other strains belonging to the same phagovar are differentiated by the PCR tests. As has been argued previously,[26] PCR fingerprinting provides additional discrimination over that provided by phage typing.

The participants who detected the smallest number of types (n=16) clustered 37 of 40 epidemiologically linked strains (including the NH1 and NH2 strains) correctly; however, 8 of the 20 unrelated isolates could not be distinguished from strains within the outbreak groups. When the dataset displaying the maximal number of PCR types (n=30) was evaluated, 29 of 40 strains were clustered. This is not an improvement when compared with the least discriminative data. Among the 20 unrelated strains, six genotypes were detected which

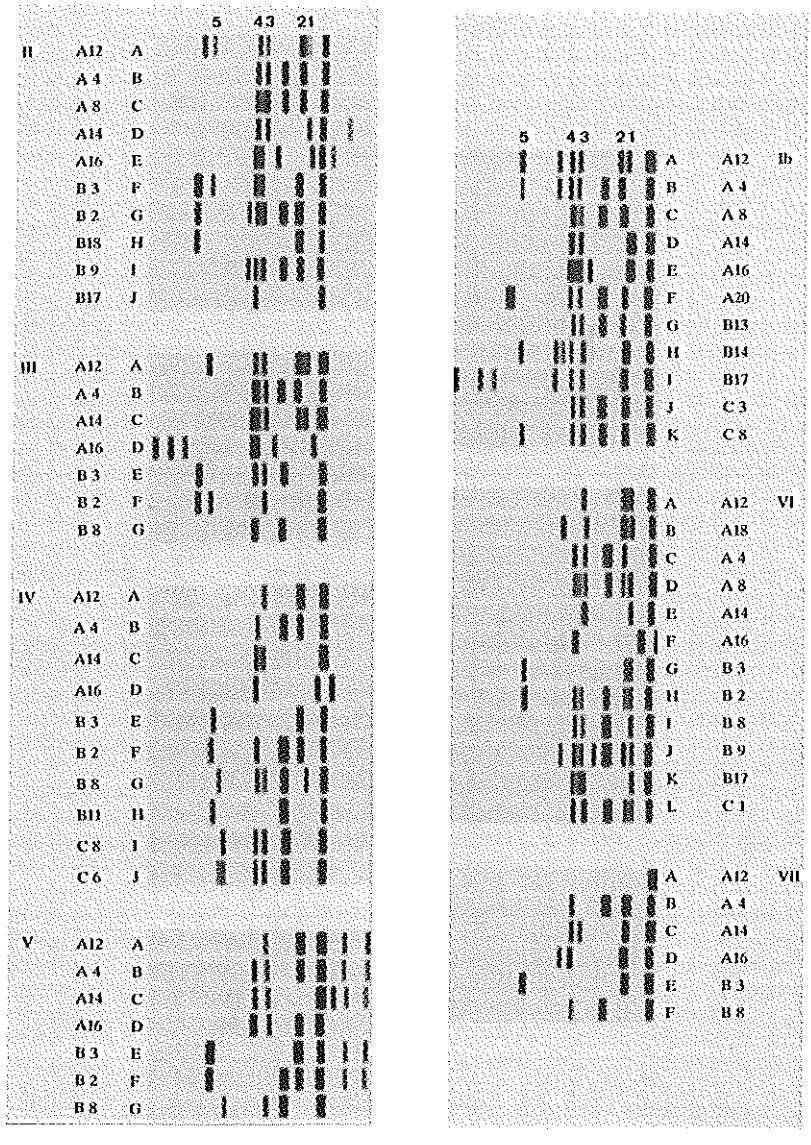

Figure 2 *Survey of unique PCR fingerprints of staphylococcal DNA by primer 7. In panel Ib through VII a survey of the banding patterns as observed by the various participants is shown. Strain numbers and PCR genocodes are indicated alongside the panels as in Table 1. Note that the migration of DNA fragments displayed in panels Ib, VI and VII is differing from those in panels II to V. For comparison, some of the common bands are identified on top with a number (1 through 5). Dataset Ia has not been included in this comparison, in case of dataset VII pattern B' has been omitted.*

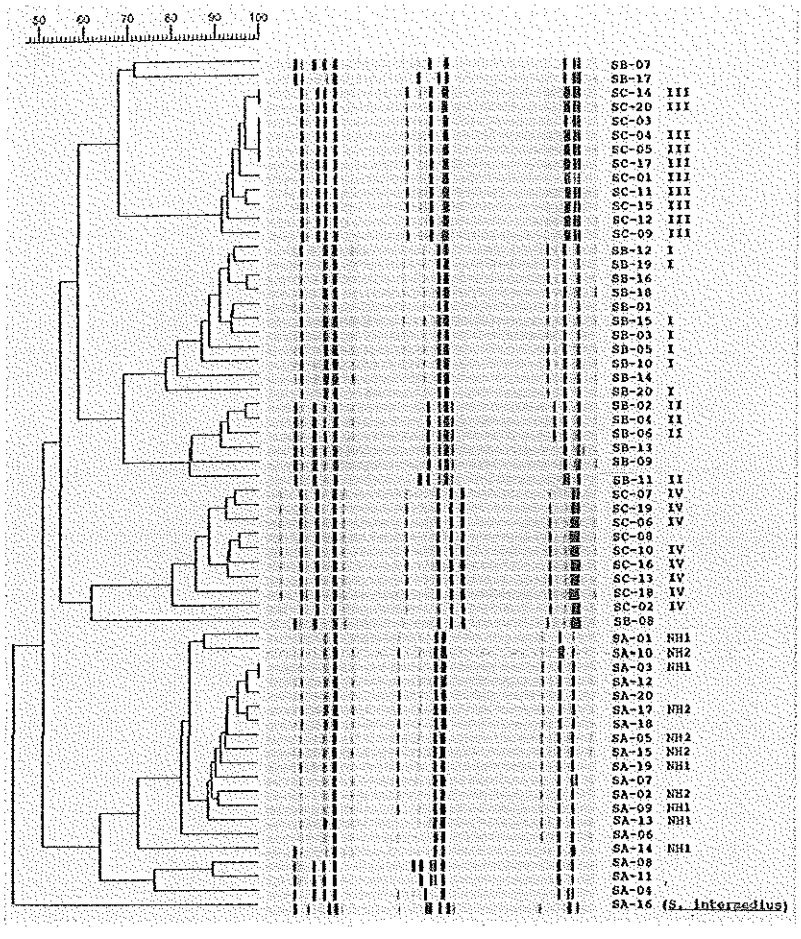

Figure 3 *Gelcompar analysis of dataset Ia. The pictures shown in Figure 1 have been digitised by scanning procedures. All three AP-PCR DNA banding patterns have been combined into one single lane. Degree of homology was subsequently calculated using Dice comparisons and correlation coefficients were determined by UPGMA analysis. On the right the strain code as presented in Table 1 is shown, as is the deduced three digit genocode (column Ia, Table 1) and the epidemiological clustering.*

were also found among the epidemiologically linked strains. Apparently, the rise in absolute number of detectable PCR genotypes adversely affects the correlation with the epidemiological data.

Comparison with pulsed field gel electrophoresis. PFGE is currently considered to be one of the most reliable and reproducible typing procedures, allowing the detection of a high degree of DNA polymorphism (15). PCR and PFGE data were compared; the results are described in Table 4. First, the PCR codes for the two groups detecting the largest (dataset IV) and the smallest (dataset Ib) number of types were simplified. The three-letter code was condensed into a single digit, and new types were defined only when more than one individual AP-PCR assay gave a different result. In case of a single change (from AAA to

AAC, for instance) subtypes were defined. The results for set Ib were rearranged into 11 types and 5 subtypes, and the data obtained for set IV defined 15 types and 9 subtypes. PFGE recognized 11 types (A through K) and 5 subtypes, equalling the numbers detected in the set Ib experiments. Nearly full epidemiological agreement exists between these latter data and PFGE results. The only difference occurs for the NH1 outbreak: PFGE found three instead of the expected two types (isolate 14 is also recognised as a deviant type by other procedures). The set IV data subdivide strains from outbreak I and IV and, as such, gave rise to an overestimation of the number of types.

Gelcompar analysis. Gelcompar analysis of the results was disappointing. Of eight datasets, only four were accessible to scanning reproduction. Of these four datasets only one could be used for successful phylogenetic analysis. The other three composite pictures could not be analyzed because of lack of contrast, excessive smiling of the gels, and low-resolution photography. In the single instance in which an interpretable phylogenetic tree could be constructed, it appeared that the result was in agreement with visual inspection and epidemiological data (Figure 3). The four sets of outbreak-related strains were clustered with homology percentages from 79 up to 93%, when data gathered with the three primers were combined. Clearly, Gelcompar analysis is heavily influenced by electrophoretic and photographic artifacts.

DISCUSSION

Approximately five years ago, PCR-mediated procedures enabling genome scanning by random amplification of polymorphic DNA were discovered.[30,31] AP-PCR can be used for genetic characterizations and comparisons even among closely related bacterial species and isolates.[1,20,21,24-26,28,29] The procedure is used with increasing frequency, facilitated by general applicability and high speed. However, only a limited number of studies have compared the effectiveness of AP-PCR typing with that of other microbiological typing procedures.[4,7,16,19-21, 25,26] In the field of staphylococcal typing, numerous studies describe conventional or molecular elucidation of clonality or epidemiological relatedness. Recently, this was combined in a comparative study on typing of a large panel of *S. aureus* isolates.[22] The overall conclusion from the present data is that AP-PCR adequately clusters strains isolated from given outbreaks. On the other hand, considerable differences between the results from different laboratories have been encountered. This is reflected by the number of isolates that are identified by a unique genotype. This number varies from 9 to 16, and participants who detect more than one type among epidemiologically clustered strains score relatively high in this respect. It has to be emphasised that during this study, several of the experimental parameters were standardized. In this respect, it is noteworthy that a relatively high degree of heterogeneity between laboratories was encountered as a result of this limited number of additional variables (Table 2). Including the DNA isolation protocol in the multicenter approach would most probably have led to an even lower degree of interlaboratory reproducibility.

In the single multicenter AP-PCR typing study that has been described to date,[17] the time- and cost-effectiveness of PCR typing were investigated. This study demonstrated that successful AP-PCR depends heavily on the optimal use of PCR protocols.[5] For this reason, it was decided not to study the intralaboratory reproducibility of the AP-PCR tests. These items have been addressed in previous studies.[7,21,23-26] However, the fact that epidemiological clusters of strains generate identical DNA-banding patters upon DNA amplification is evidence of at least a reasonable degree of intralaboratory reliability. Upon reamplification of some of the DNA samples, as performed in two of the participating laboratories, AP-PCR profiles appeared to be reproducible.

Table 4: *Comparison of PCR fingerprinting and PFGE on the basis of simplified genetic codes for the PCR assay.*

	PCR code		Simplified code		
Strain	Ib	IV	Ib	IV	PFGE code
A16	FEF	FDF	1	1	I
A4	BBB	BBB	2	2	E
A12	AAA	AAA	3	3	J
A18	AAA	AAA	3	3	J
A20	AAA	AAA	3	3	J
A6	AAC	AAA	3a	3	C
A7	AAC	CAA	3a	3a	B
A8	CCD	DBC	4	4	G
A11	CCD	DBD	4	4a	F
A1	AAA	AAA	3	3	K1
A9	AAA	AAA	3	3	K2
A3	AAA	AAA	3	3	A
A13	AAA	AAA	3	3	A
A14	EDE	-CE	5	5	H
A19	AAA	AAA	3	3	K3
A17	AAA	AAA	3	3	A
A2	AAA	AAA	3	3	A
A15	AAA	AAA	3	3	A
A5	AAA	AAA	3	3	A
A10	DAA	EAA	3b	3b	D
B7	BBB	BBH	2	2a	D
B3	AAA	AE-	3	3c	A
B5	AAA	AEA	3	3c	A
B10	AAA	AEA	3	3c	A
B12	AAA	AEL	3	6	A1
B15	AAA	AEL	3	6	A
B19	AAA	AEL	3	6	A
B20	AAA	AEL	3	6	A
B1	AAA	AEA	3	3c	A1
B16	AAA	AEL	3	6	A1
B18	AAA	AEK	3	6	A
B17	JIM	LCO	6	7	E
B14	AHL	AAN	7	3d	A2
B8	HBI	IGI	8	8	F
B2	GFH	GFG	9	9	B
B4	GFH	HFG	9	9a	B
B6	GFH	HFG	9	9a	B
B11	GFK	KHK	9a	10	C
B9	IDJ	JBJ	10	11	B
B13	GGJ	RBM	11	12	B1
C3	BJB	OBP	2a	13	C
C1	BJB	MBP	2a	13a	A
C4	BJB	MBP	2a	13a	A
C5	BJB	-BP	2a	13a	A
C9	BJB	MBP	2a	13a	A
C11	BJB	MBP	2a	13a	A
C12	BJB	MBP	2a	13a	A
C14	BJB	MBP	2a	13a	A
C15	BJB	MBP	2a	13a	A
C17	BJB	MBP	2a	13a	A
C20	BJB	MBP	2a	13a	A
C8	HKI	PII	2a	14	B1
C2	HKI	NII	8a	14a	B
C6	HKI	PJI	8a	15	B
C7	HKI	PJI	8a	15	B
C10	HKI	-II	8a	14a	B
C13	HKI	NII	8a	14a	B
C16	HKI	PJI	8a	15	B
C18	HKI	PJI	8a	15	B
C19	HKI	PII	8a	14	B

The data sets Ib and IV have been simplified by changing three- into one-letter codes. New types were defined when at least two characters from the three letter code had changed; single assay changes result in subtyping (a through d). Epidemiologically related clusters are indicated by returns, strain numbering is as in Table 1.

Reproducibility was affected by the nature of the primer used and the identity of the intratube thermoprofile. Isolates SA-04, SB-07 and SC-03, all of which were *S. aureus* ATCC 12600, were included in the three sets of strains to evaluate the reproducibility of AP-PCR. Only one of the participating laboratories unequivocally identified all three strains to belong to a single genotype. It must be emphasised that the other typing procedures also detected gross differences among these three particular isolates. It has been demonstrated previously that genetic variability as measured by PCR can be a consequence of repeated conservation and "reviving" of strains; this is probably due to replication defects or the absence or presence of lytic phages.[3] This might be an explanation for the extensive variability encountered among the ATCC strains. PFGE, for instance, corroborates the PCR data in six of seven PCR datasets by designating genotypes E, D and C. Plasmid types are also very different: B, D and C are the indexes. This, in combination with other experimental results, may be indicative of intrastrain heterogeneity or sampling error. Computerised correction of AP-PCR artifacts is currently under development.[13, 14] It is particularly important to implement this approach, which takes reproducibility and erroneous amplification into account, when multicenter studies are performed. However, on the basis of the results of the present study, it is expected that interinstitute standardisation will be very hard to achieve.

PCR typing is currently restricted to laboratories with appropriate equipment and experimental expertise. In this respect, the applicability of AP-PCR is as yet limited. It is clear from the present study that generation and interpretation of PCR data is likely to vary among laboratories. The percentage of variant types identified can be on the order of 27 to 50%, based on the application of three PCR tests and a single DNA-processing protocol. The variables that still exist between laboratories (Table 2) must be responsible, at least in part, for these large discrepancies. From the duplicate experiments performed in Rotterdam, it was concluded that gel electrophoresis is a major cause of experimental variability; this has been confirmed by a recent report.[10] An increase in electrophoresis time led to improved separation, which in turn enabled successful digitization and Gelcompar analysis. It is also acknowledged that the present study suffers from the fact that DNA isolation and primer quality were standardized. If this had not been the case, differences between laboratories may have been even larger.

AP-PCR shares characteristics with the genome-scanning capacities of electrophoretic techniques such as PFGE and FIGE. These last two procedures identify epidemiological relations for staphylococci that are in good agreement with the present data (Table 4). Recently, guidelines for interpretation of PFGE patterns for outbreak investigations were proposed by an American working group.[8] Since these rules have been used to define staphylococcal subtypes in the collection used in the previous and present studies,[22] our PCR data may enable the development of similar rules for the definition of PCR subtypes. The rules should be based either on differences within the banding pattern generated during a single PCR or on differences in composite genocodes as presented in Table 1 and simplified in Table 4. The present study indicates that differences in the combined results of multiple AP-PCR assays are better indicators of genetic variability than the results of individual assays. It is clear, however, that PFGE subtypes do not fully match the subtypes as defined by PCR. It is advisable to perform model studies starting with PFGE-uniform strains on the one hand and PCR-uniform strains on the other hand. For studies such as these it is also important to start the analysis with a collection comparable to the set of strains in this study: it should provide a mixture of unique, solitary isolates together with epidemiologically well-defined clusters.

Our data demonstrate that PCR fingerprinting deserves a position among the procedures that are well suited for the epidemiological analysis of *S. aureus*. The procedure seems particularly appropriate for the high-speed typing of nosocomial isolates. This conclusion

was also drawn previously;[2] on the basis of theoretical considerations, it was suggested that AP-PCR is a cost-effective procedure as well. It is necessary to test multiple primers, since differences in discriminatory power are to be expected. Strain-specific amplicons can be generated quite easily, even among clonally related isolates of *S. aureus*. With the exclusion of subtype numbering for the other typing strategies, AP-PCR generates the largest number of individual types. It exceeds the resolution of PFGE, which detects 11 types and 5 subtypes. Only phage typing and RFLP mapping approach the average number of types detectable by AP-PCR. It must be emphasized, however, that the generation of excessive numbers of types introduces the possibility that the relationship between the AP-PCR data and epidemiological characteristics will deteriorate.

Finally, we recommend establishing collections like the one used in this and the previous study [22] for other microorganisms as well. The availability of these strains enables the individual researcher to establish the value of newly developed typing tools or to use these strains as internal controls in typing studies. Well-documented collections or experimental protocols can be used for standardization of typing procedures,[27] an initiative important for the development of international standards on genetic relatedness or clonality among pathogenic microorganisms.

REFERENCES

1 **Akopyanz N, Bukanov NO, Westblom TU, Krurovich S and Berg DE.** DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. *Nucleic Acids Res*1992;**20**:5137-42.

2 **Bingen E.** Applications of molecular methods to epidemiologic investigations of nosocomial infections in a pediatric hospital. *Infect Control Hosp Epidemiol* 1994;**15**:488-93.

3 **Brikun I, Suziedelis K and Berg DE.** DNA sequence divergence among derivatives of *Escherichia coli* K-12 detected by PCR-based RAPD fingerprinting. *J Bact* 1994;**176**:1673-1682.

4 **Brousseau R, Saint-Onge A, Prefontaine G, Masson L and Cabana J.** Arbitrary primer polymerase chain reaction, a powerful method to identify *Bacillus thuringiensis* serovars and strains. *Appl Environ Microbiol* 1993;**59**:114-9.

5 **Caetano-Anolles G, Bassam BJ and Gresshoff PM.** Buffer components tailor DNA amplification with arbitrary primers. *PCR Meth Appl* 1994;**4**:59-61.

6 **Centers for Disease Control** Postsurgical infections associated with an extrinsically contaminated intravenous anaesthetic agent - California, Illinois, Maine, Michigan, 1990. *Morbid Mortal Weekly Rep* 1990;**39**:426-33.

7 **Elaichouni A, Verschraegen G, Claeys G, de Vleeschouwer M, Godard C and Vaneechoutte M.** *Pseudomonas aeruginosa* serotype O12 outbreak studied with arbitrary primer PCR. *J Clin Microbiol* 1994;**32**:666-71.

8 **Goering RV.** Defining inter-isolate relationships between bacterial isolates by use of PFGE of DNA macrorestriction fragments. Abstr. Int. Meeting on Bacterial Epidemiological Markers. Cambridge, UK, April 1994, abstract F19.

9 **Goetz MB, Mulligan ME, Kwok R, O'Brien H, Caballes C and Garcia JP.** Management and epidemiologic analyses of an outbreak due to methicillin-resistant *Staphylococcus aureus*. *Am J Med* 1992;**92**:607-14.

10 **He G, Prakash CS, Jarret RL, Tuzun S and Qiu J.** Comparison of gelmatrices for resolving PCR amplified DNA fingerprint profiles. *PCR Meth Appl* 1994;**4**:50-1.

11 **Jarvis WR.** Usefulness of molecular epidemiology for outbreak investigations. *Infect Contr Hosp Epid* 1994;**15**:500-3.

12 **Kloos WE and Lambe WD jr.** Staphylococcus, p. 222-227. *In* A. Balows, W.J. Hausler, K.L. Herrmann, H.D. Isenberg and H.J. Shadomy (Ed) Manual of Clinical Microbiology, 5th edition, American Society for Microbiology, Washington, D.C 1991.

13 **Lamboy WF.** Computing genetic similarity coefficients from RAPD data: the effects of PCR artifacts. *PCR Meth Appl* 1994;**4**:31-7.

14 **Lamboy WF.** Computing genetic similarity coefficients from RAPD data: correcting for the effects of PCR artifacts caused by variation in experimental conditions. *PCR Meth Appl* 1994;**4**:38-43.

15 **Maslow JN, Mulligan ME and Arbeit RD.** Molecular epidemiology: the application of contemporary techniques to typing bacteria. *Clin Infect Dis* 1993;**17**:153-62.

16 **Mazurier S, Audurier A, Marquet-Van der Meer N, Notermans S and Wernars K.** A comparative study of randomly amplified polymorphic DNA analysis and conventional phage typing for epidemiological studies of *Listeria monocytogenes* isolates. *Res Microbiol* 1992;**143**:507-12.

17 **Penner GA, Bush A, Wise R, Domier L, Kasha K, Laroche A, Scoles G, Molnar SJ and Fedak G.** Reproducibility of random amplified polymorphic DNA RAPD analysis among laboratories. *PCR Meth Appl* 1993;**2**:341-5.

18 **Pfaller MA, Wakefield DS, Hollis R, Fredrickson M, Evans E and Massanari RM.** The clinical microbiology laboratory as an aid in infection control. The application of molecular techniques in epidemiologic studies of methicillin-resistant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* 1991;**14**:209-17.

19 **Ralph D, McClelland M, Welsh J, Baranton G and Perolat P.** *Leptospira* species categorised by arbitrarily primed polymerase chain reaction (PCR) and by mapped restriction polymorphisms in PCR-amplified rRNA genes. *J Bacteriol* 1993;**175**:973-81.

20 **Saulnier P, Bourneix C, Prevost G and Andremont A.** Random amplified polymorphic DNA assay is less discriminant than pulsed field gelelectrophoresis for typing strains of *Staphylococcus aureus*. *J Clin Microbiol* 1993;**31**:982-5.

21 **Struelens M, Bax R, Deplano A, Quint W and van Belkum A.** Concordant clonal delineation of methicillin-resistant *Staphylococcus aureus* by macrorestriction analysis and polymerase chain reaction genome fingerprinting. *J Clin Microbiol* 1993;**31**:1964-70.

22 **Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S, Goering R, Hancock G, Hebert GA, Hill B, Hollis R, Jarvis WR, Kreiswirth B, Eisner W, Maslow J, McDougal LK, Miller JM, Mulligan M and Pfaller MA.** Comparison of traditional and molecular methods of typing of *Staphylococcus aureus*. *J Clin Microbiol* 1994;**32**:407-15.

23 **Van Belkum A.** DNA fingerprinting of medically important microorganisms using the polymerase chain reaction. *Clin Microbiol Rev* 1994;**7**:174-84.

24 **Van Belkum A and Meis J.** Polymerase chain reaction mediated DNA fingerprinting in bacterial epidemiology. *Clin Infect Dis* 1994;**18**:1017-8.

25 **Van Belkum A, Bax R and Prevost G.** Comparison of four genotyping assays for epidemiological study of methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* 1994;**13**:420-4.

26 **Van Belkum A, Bax R, Peerbooms P, Goessens W, van Leeuwen N and Quint W.** Comparison of phage typing and DNA fingerprinting by PCR for discrimination of methicillin resistant *Staphylococcus aureus* isolates. *J Clin Microbiol* 1993;**31**:798-803.

27 **Van Embden JDA, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans PWM, Martin C, Mcadam R, Shinnick TM and Small PM.** Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardised methodology. *J Clin Microbiol* 1992;**31**:406-9.

28 **Versalovic J, Koeuth T and Lupski JR.** Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 1991;**19**:6823-31.

29 **Welsh J, Pretzman C, Postic D, Saint-Girons I, Baranton G and McClelland M.** Genomic fingerprinting by arbitrarily primed polymerase chain reaction resolves *Borrelia burgdorferi* to three distinct phyletic groups. *Int J Syst Bacteriol* 1992;**42**:370-7.

30 **Welsh J and McClelland M.** Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res* 1990;**18**:7213-8.

31 **Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV.** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 1990;**18**:6531-5.

CHAPTER FOUR
MOLECUR BIOLOGICAL TYPING METHODS

PART TWO
FOOD-INITIATED OUTBREAK OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ANALYZED BY PHENO- AND GENOTYPING

Jan Kluytmans, Willem van Leeuwen, Wil Goessens, Richard Hollis, Shawn Messer, Loreen Herwaldt, Hajo Bruining, Max Heck, Jules Rost, Nan van Leeuwen, Alex van Belkum and Henri Verbrugh.

Adapted from: *J Clin Microbiol* 1995;**33**:1121-8

SUMMARY

An outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) involving 27 patients and 14 healthcare workers (HCWs) was studied. The outbreak started in the hematology unit of the University Hospital Rotterdam, Dijkzigt, The Netherlands, and spread to the surgical unit. Twenty-one patients (77.8%) developed clinical disease, and five died. Subsequently, MRSA was detected in food and in the throat of one of the HCW who prepared food for hematology patients. Food contaminated by an HCW most likely caused the first case of MRSA septicemia. This route of transmission has not been described before. The outbreak strain was probably transmitted to the surgical unit by a colonized nurse, where it caused an explosive outbreak. Airborne MRSA transmission played an important role in disseminating the organism. The outbreak was controlled within six months by intensifying surveillance, temporarily closing the affected wards, treating carriers, and instituting an MRSA ward outside the hospital.

Phage typing, insertion sequence probing, protein A gene typing and DNA fingerprinting by PCR revealed that all outbreak-related isolates were identical. By pulsed field gel electrophoresis, all but one of the outbreak-related isolates were determined to be identical. Protein A gene typing identified numerous (11) repeat units in all outbreak-related isolates, which supports that the outbreak strain may have been more virulent and more transmissible than other MRSA strains. Pheno- and genotypic typing studies underlined the value of DNA fingerprinting methods for investigation of MRSA epidemiology. Optimal discriminatory power was achieved by combining the results of four genotyping methods.

INTRODUCTION

In Europe, the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals varies considerably from country to country. Generally, the incidence of MRSA increases from north to south. In a pan-European surveillance, the incidence was found to be highest in Spain, France and Italy (30.3 to 34.4%) and lowest in Denmark, Sweden and The Netherlands (0.1 to 1.5%).[34] Despite the low incidence in Dutch hospitals, outbreaks of MRSA occur regularly, especially in the larger teaching hospitals. Most outbreaks are due to the import of strains by patients transferred from hospitals in countries with increased MRSA incidence.[33] Outbreaks in low prevalence settings offer unique opportunities to determine the value of various typing methods, since the clonal relatedness among the MRSA isolates involved is obvious.

Scientists still debate whether one clone of MRSA has spread throughout the world.[1,17,21] However, numerous investigations have demonstrated that the phenotype[19,21,30] and genotype[7,13,14,17,28] of MRSA vary. These procedures are particularly useful for comparing bacterial isolates involved in nosocomial outbreaks. Recently, it has been shown that especially the genotypic assays posses excellent discriminatory power and exhibit subtle resolution capabilities.[29]

This report describes the epidemiological aspects of an MRSA outbreak which occurred in the University Hospital Rotterdam, The Netherlands. The initial reservoir for the outbreak strain was probably a dietary worker, who carried MRSA in his throat, and prepared food for patients on the hematology unit. Transmission of MRSA by contaminated food has not been described before.

MATERIALS AND METHODS

Isolation and processing of MRSA strains. Between November 1992 and April 1993, an outbreak of MRSA occurred in the University Hospital Rotterdam. MRSA strains were isolated from 27 patients (Table 1), 14 healthcare workers (HCWs) and from environmental samples. Surveillance cultures were routinely made for patients specimens from the nares, throat, perirectal area and, if present, skin lesions (including wounds) and catheter insertion sites. For patients who had urinary catheters, urine was also cultured. Surveillance cultures from HCW consisted routinely of swabs from the nares and from skin lesions if present.

The initial cultures were inoculated on Columbia agar base supplemented with 5% sheep blood and mannitol salt agar. The plates were incubated at 37°C for 48 hours and inspected after 18 to 24 h and after 42 to 48 h. All isolates were identified on the basis of colony morphology, positive catalase slide test, and coagulase tube-tests. Additionally, biochemical analysis was performed with Vitek® (BioMerieux, Lyon, France). Methicillin resistance was determined by inoculation of strains on Mueller Hinton agar (Oxoid CM 337, Brunschwig, Amsterdam, The Netherlands) with a disk containing 5 µg of methicillin (Oxoid, Brunschwig). Plates were incubated at 30°C for 24 h, and inhibition zones were measured.[3] Isolates with inhibition zones smaller than 17 mm diameter were considered methicillin-resistant. All strains were grown as monocultures in brain heart infusion (BHI) broth and stored as lyophilised powder.

A sample of 17 outbreak-related isolates, including isolates from patients, HCWs and the environment were evaluated by several typing methods (outbreak group; see Table 2). Thirteen epidemiologically unrelated strains were included in the typing studies as comparison strains: five isolates were obtained in the same hospital from patients transferred from foreign hospitals (control group 1), and eight isolates with the same phagovar as the

Table 1: *Clinical, geographical and bacteriological data for patients colonized or infected with MRSA.*

Patient no.	ward	MRSA isolation date (mm/dd/yy)	week of outbreak	clinical diagnosis or surgery
1	hematology	11/12/92	1	acute myeloid leucemia
2	vascular surgery	11/22/92	3	atherosclerotic vascular disease
3	vascular surgery	11/24/92	4	atherosclerotic vascular disease
4	vascular surgery	11/26/92	4	atherosclerotic vascular disease
5	vascular surgery	11/26/92	4	atherosclerotic vascular disease
6	vascular surgery	11/26/92	4	atherosclerotic vascular disease
7	vascular surgery	11/26/92	4	atherosclerotic vascular disease
8	vascular surgery	11/26/92	4	aneurysm of aorta
9	vascular surgery	11/28/92	4	atherosclerotic vascular disease
10	vascular surgery	12/01/92	5	atherosclerotic vascular disease
11	vascular surgery	12/03/92	5	aneurysm of aorta
12	surgical ICU	12/03/92	5	peritonitis
13	general surgery	12/03/92	5	osteomyelitis
14	vascular surgery	12/07/92	6	atherosclerotic vascular disease
15	vascular surgery	12/07/92	6	aneurysm of aorta
16	vascular surgery	12/07/92	6	atherosclerotic vascular disease
17	surgical ICU	12/21/92	8	colon surgery for obstruction
18	surgical ICU	03/01/93	18	liver transplantation
19	general surgery	03/03/93	18	esophageal cancer
20	vascular surgery	03/03/93	18	aneurysm of aorta
21	general surgery	03/06/93	18	colon cancer
22	general surgery	03/10/93	19	breast cancer
23	vascular surgery	03/15/93	19	atherosclerotic vascular disease
24	general surgery	03/17/93	20	colonic diverticulosis
25	vascular surgery	03/22/93	20	atherosclerotic vascular disease
26	general surgery	03/30/93	22	colon cancer
27	general surgery	03/31/93	22	gastric cancer

+ Y, yes; N, no.
* defined as mortality 1 year after MRSA was first isolated.
In patients 1, 2, and 18, mortality was directly related to infection with MRSA.

Table 1

presence of infection[+]	site of infection	prosthetic vascular graft[+]	amputation[+]	outcome[*]
Y	blood	N	N	died
Y	wound	Y	N	died
Y	wound	Y	Y	survived
Y	wound	N	N	survived
Y	wound	N	Y	survived
Y	wound	N	Y	survived
Y	wound	Y	Y	survived
N		Y	N	survived
Y	wound	Y	Y	survived
Y	wound	Y	N	survived
N		Y	N	survived
Y	wound	N	N	survived
Y	wound	N	N	survived
Y	wound	N	Y	survived
Y	wound	Y	N	survived
Y	wound	Y	Y	survived
Y	wound	N	N	died
Y	wound	N	N	died
Y	wound	N	N	survived
Y	wound	Y	N	survived
Y	wound	N	N	survived
Y	wound	N	N	survived
Y	wound	Y	N	survived
Y	wound	N	N	survived
Y	wound	Y	Y	died
Y	wound	N	N	survived
N		N	N	survived

outbreak-related isolates were obtained from the Dutch National Institute for Public Health and Environmental Protection, Bilthoven, The Netherlands, (control group 2).

Antimicrobial susceptibility testing. In addition to susceptibility to methicillin, the susceptibility of the strains to ciprofloxacin, erythromycin, oxacillin, rifampicin, tetracycline, phosphomycin, tobramycin, co-trimoxazole, clindamycin, vancomycin and fusidic acid were determined. All assays were performed by the method of Bauer *et al.*,[3] using breakpoints according the National Committee for Clinical Laboratory Standards guidelines.[22] Resistance was classified as 0, intermediate susceptibility as 1, and full susceptibility was clasified as 2.

Phage typing. Phage typing was performed at the Dutch National Institute of Public Health and Environmental Protection, by application of the international set of typing phages and a set of typical Dutch phages.[18,23] Different phage patterns were given a type designation.

PCR-mediated DNA fingerprinting (arbitary primed PCR [AP-PCR]). AP-PCR was performed essentially as described before.[30] DNA was isolated from overnight cultures by lysostaphin treatment, guanidine isothiocyanate-lysis,[5] and subsequent DNA affinity chromatography. Approximately 5 ng of DNA was included per PCR mixture. The PCR mixture further consisted of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton-X100. Deoxyribonucleotide triphosphates were present at 0.2 mM in reaction mixture, and 0.5 U of *Taq* DNA polymerase (Sphaero Q, Leiden, The Netherlands) was included. Five differrent primers were included in the typing assays. The designations and sequences of the primers were as follows:

- 186: (5'-GGTTGGGTGAGAATTGCACG-3');
- 188: (5'-AAGAGCCCGT-3');
- 192: (5'-GTGGATGCGA-3');
- ERIC1: (5'-ATGTAAGCTCCTGGGGATTCAC-3');
- ERIC2: (5'-AAGTAAGTGACTGGGGTGACGC-3').

All primers were applied in separate assays (50 pmol of primer per PCR mixture). The amplification was performed in a model 60 thermocycler (Biomed, Thores, Germany) by a program consisting of 40 repeated cycles of 1 min at 94°C, 1 min at 25°C, and 2 min at 74°C. Prior to cycling, the mixtures were denatured at 94°C for 4 min, and postcycling, the mixtures were incubated at 74°C for an additional 10 min. PCR samples were analysed by agarose gel electrophoresis. The gels, containing 2% agarose in 40 mM Tris-borate (pH 7.8)-2 mM EDTA (0.5 X TBE), were run in the presence of ethidium bromide. After photography (Polaroid high-speed sheet film 57), DNA fingerprints were compared by visual inspection.

The results for each primer were indexed by numbering, thereby defining the number of different DNA fingerprints (i.e. amplicon banding patterns) that could be distinguished by single assays. One- and two-band differences between two DNA fingerprints derived from different strains were classified as subclonal variation, indicated by adding a prime to the index character (see Table 2). The overall PCR type was defined by a combination of the results obtained with the five individual primers. If subclonal variations were detected with individual primers, this was indicated by subnumbering of the PCR type.

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed at the University of Iowa, Iowa City. Contour-clamped homogeneous electric field (CHEF) analysis was performed using the CHEF DR-II (Bio-Rad Laboratories, Richmond, Calif.). Bacteria were embedded in low-melting-temperature agarose (FMC Corp., Philadelphia, Pa.) and treated with lysostaphin and proteinase K-sodium dodecyl sulfate (SDS) according to established protocols.[24,28,29] DNA was digested in situ by *Sma*I (New England Biolabs, Beverly, Mass.) according to the manufacturer's instructions. Agarose gels (1%) were run in 0.5XTBE at 13°C and at 6 V/cm. Switching times were ramped from 10 to 90 s for a total run time of 24 h for optimal

separation of DNA fragments between 50 and 500 kB in size. Strain differences were based on the detection of restriction fragment length polymorphism (RFLP). One- and two-band differences were designated subclonal variations, indicated by subnumbering; differences of three or more bands were interpreted as indicating different strains. Comparisons and calculation of the percent homology were performed manually. Dendrograms were constructed by the unweighted pair group method using arithmetic averages (UPGMA) subroutine of the PHYLIP Software.[9,11]

Detection of protein A gene polymorphisms by PCR. Length polymorphisms in the staphylococcal protein A gene were determined by PCR essentially as described before.[12] In short, the repetitive region within the protein A gene was amplified by using oligonucleotide primers with the following DNA sequences:

- 5'-TGTAAAACGACGGCCAGTGCTAAAAAGCTAAACGATGC-3' and
- 5'-CAGGAAACAGCTATGACCCCACCAAATACAGTTGTTACC-3'.

After PCR, DNA was cleaved with the restriction enzyme *Rsa*I (Boehringer GmbH, Mannheim, Germany) and RFLPs were determined by electrophoresis in 2% agarose gels run in 0.5XTBE. The number of repetitive units present in the genes' variable region was estimated by comparisons with molecular weight markers (100-bp marker, Pharmacia, Gouda, The Netherlands).

Insertion sequence probing. Insertion sequence probing was performed at the Dutch National Institute of Public Health and Environmental Protection. For RFLP analysis, extraction of chromosomal DNA and Southern blotting were performed as described previously.[15] Genomic DNA was extracted from 1.5 ml overnight culture by using lysostaphin, SDS, and proteinase K. DNA was purified further by extraction with phenol-chloroform and ethanol precipitation. Finally, the DNA was dissolved in 100 µl TE (10mM Tris-HCL, 1 mM EDTA, pH 8.0). *Cla*I (Boehringer) was used to digest DNA according to the manufacturer's instructions.[4] The digested DNA was analysed by electrophoresis in a 1% agarose gel at 25 V overnight in TBE (89 mM Tris-HCl, 89 mM boric acid, 25 mM EDTA, pH 8.2) and stained in ethidium bromide (0.5 µg/ml). Southern blots were prepared on hybond N⁺ membranes (Amersham International, Amersham, United Kingdom) by using a vacuum miniblot system (Millipore Corp., Bedford, Mass.) and stored at 4°C until use. For hybridization, a probe based on the IS*431* sequence, an insertion-like element which is found frequently among staphylococci,[2] was used. The probe, designed to amplify an 800-bp sequence from IS*431*, was produced by PCR using two primers:

- primer 1, 5'-TACATCATGTTAATAAGGG-3' and
- primer 2, 5'-TTGCGTGAGTGTGGCGAAGC-3'

The target for amplification was 5 µl of *S. aureus* suspension which was also used for the digestion as described above. Amplification was performed as described previously [25] for 35 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C using a thermocycler (Perkin-Elmer). After amplification the PCR products were divided into aliquots of 10 µl and purified by agarose gel electrophoresis using low-melting-temperature agarose (preparative grade, Biorad).[26] After the DNA-fragments were seperated, agarose blocks containing DNA with the expected molecular weight were cut from the gel and directly used for labelling or stored at -20°C until they were processed. Nonradioactive labelling of probe DNA with horseradish peroxidase was performed directly in the agarose blocks by using the enhanced chemiluminescence gene detection system (Amersham International) according to the manufacturer's instructions. One agarose block containing labeled DNA was used for hybridization of one blot. Southern Blot hybridization was performed and recorded as described previously.[1]

RESULTS

Epidemiological surveillance. The first patient involved in the MRSA outbreak was identified in the hematology unit, were she was treated for acute myeloid leukemia. Because of neutropenia (granulocyte count, <500 X 10^9/liter), she was nursed in a private room with laminar airflow high-efficiency particulate air filtration, as were all other neutropenic patients on this unit. The patient was given oral ciprofloxacin prophylactically, as was the unit's routine. Subsequently, she developed sepsis with MRSA of which she died within three days, despite immediate treatment with vancomycin. The surveillance cultures, which were obtained twice weekly, were negative until the patient developed the bacteremia. Also, two sets of surveillance cultures which were obtained for all other patients and the HCWs and other contacts on the hematology unit, were all negative for MRSA. No environmental source was identified.

Ten days after the first patient was identified, additional patients who carried MRSA were identified on the surgical unit, which was located far from the hematology unit. A nurse on the surgical unit was colonized by a MRSA strain (strain 6, Table 2) that was identical to the outbreak strain. This nurse probably transmitted the outbreak strain of MRSA to the surgical unit, as she had been transferred from the hematology unit to surgery shortly after the first patient was identified. After MRSA was detected on the surgical unit, surveillance cultures were done repeatedly for all patients and HCWs (nurses, physicians and paramedical employees) on this unit. A total of 26 patients and 13 HCWs (including the nurse who came from the hematology unit) carried MRSA. Of the 26 surgical patients colonized with MRSA, 20 (77%) developed a clinically proven MRSA infection. Four of these individuals died (25%); two of these deaths were directly related to the MRSA infection. The clinical diagnosis and geographical and bacteriological data for the 27 MRSA-postive patients are detailed in Table 1.

Figure 1 shows the incidence of MRSA-positive patients during the outbreak period. The first episode of the outbreak involved primarily patients who had had vascular surgery who had necrotic wounds or had prosthetic vascular grafts that had been implanted recently. Most of these patients developed clinically significant wound infections.

To control this outbreak, the vascular surgery ward was closed and the patients who were colonized with MRSA were transferred to an MRSA cohort isolation facility which was created outside the hospital in week number 10 after the outbreak began. A separate team of nurses and doctors took care of these patients. The surgical ward, including the floors, walls, ceillings, and furniture, was cleaned completely and disinfected using 4-chlor-2-benzylphenol (2% [wt/vol]) and *ortho*-phenylphenol (2% [wt/vol]). HCWs who were colonized with MRSA were treated with nasal mupirocin ointment (Bactroban®; SmithKline Beecham) and sent home until MRSA carriage was eradicated. As can be seen in Figure 1, the first episode was rapidly controlled. In week 13, only a single patient remained in the isolation facility. It was then decided to close this facility and treat the patient in a regular hospital department, in strict isolation (private room with negative air pressure, and gloves, gowns, and masks required for everyone who entered the room, and the patient confined to his room as much as possible). Despite strict isolation, this particular patient served as a source for the second episode of the outbreak. This episode involved fewer patients and HCWs but more surgical wards. Most patients probably acquired MRSA in the surgical intensive care unit (ICU), where they were treated for a few days postoperatively. However, MRSA carriage was not detected until after the patients were transferred to other surgical wards. The second episode of the outbreak was controlled by intensifying bacteriological surveillance of patients and HCWs and by reinstituting the isolation facility.

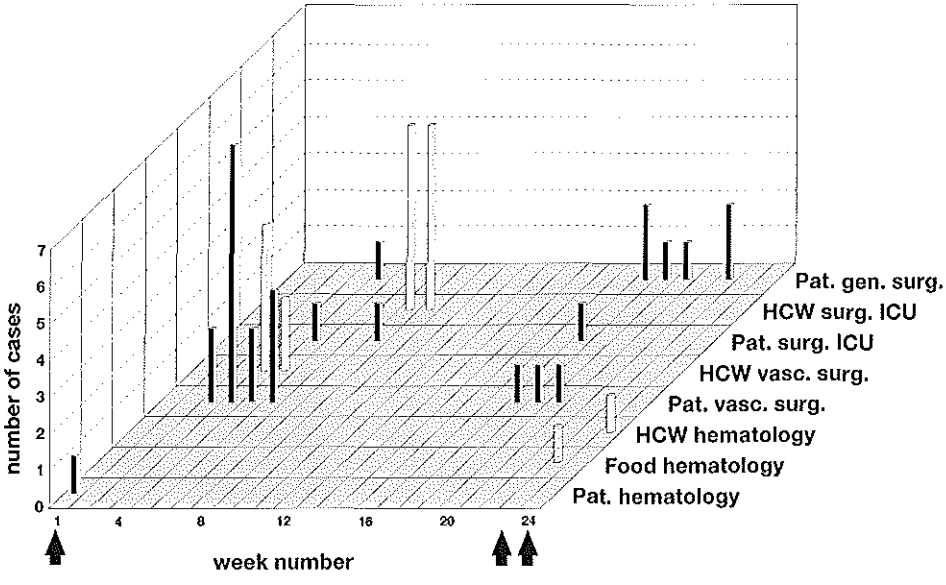

Figure 1: Incidence of MRSA in patients (Pat.), health care workers (HCW) and food during the outbreak in the Hematology unit, the Vascular Surgery unit, the Surgical ICU and the General Surgery unit. The boxed regions indicate the number of new cases per week. The arrows indicate the three strains isolated from the dietary worker, from food and from the patient in the Hematology unit.

Twenty-two weeks after the outbreak began, routine bacterial cultures of food prepared for neutropenic patients in the hematology unit revealed MRSA in a piece of banana, which had been peeled by a dietary worker who worked in this unit. Therefore, all personnel who prepared food for patients on the hematology unit were screened for MRSA carriage. MRSA was cultured repeatedly from throat swabs but not from the nares or the perirectal area of the dietary worker. This HCW was working on the hematology unit at the time the first patient was detected but never contacted directly patients or HCWs with MRSA. The MRSA strains cultured from hematology patient, the piece of banana, and the dietary worker were identical (Table 2 strains 1 a/b, 2 and 3, respectively). The dietary worker was treated succesfully by application of mupirocin nasal ointment twice a day and daily washing of body and hair with disinfecting soap for 5 consecutive days.

Antibiotic sensitivity of MRSA. All outbreak-strains that were evaluated had similar antibiograms (Table 2). Except for isolate number 17, all outbreak strains (1 through 17) were resistant to methicillin. Also all isolates were resistant to tobramycin, erythromycin, ciprofloxacin, rifampicin, clindamycin, and tetracycline. All were susceptible to vancomycin and co-trimoxazole. More variability was found regarding susceptibility to phosphomycin and fusidic acid. Isolate 17 was sensitive to methicillin as determined by the disk diffusion assay. This isolate was obtained from a patient who also carried methicillin-resistant isolates. The antibiograms of the isolates in control group 1 (Table 2, isolates 18 to 22) were more heterogeneous. The antibiograms of the isolates in control group 2 (Table 2, isolates 23 to 30) were generally similar to the outbreak strains.

Phage typing. All outbreak-related isolates, including the methicillin-susceptible isolate 17, were phage type III29. None of the isolates in control group 2 were of this phage type.

Table 2: *Comparison of results from phenotypic and genotypic typing methods.*

Group and isolation date (M/Y)	Site of source[a]	Ward	isolate no.[b]	antibiotic susceptibility[c]	phage type	protein A type[d]
OUTBREAK GROUP						
11/92	blood	hematology	1a/b	20020002020	III29	11
03/93	banana	hematology	2	20020002020	III29	11
03/93	HCW	hematology	3	20020002020	III29	11
11/92	ASP	vascular surgery	4	00020002020	III29	11
11/92	nose	vascular surgery	5a/b	00020002000	III29	11
11/92	HCW	vascular surgery	6	20020002020	III29	11
11/92	HCW	vascular surgery	7	20020002020	III29	11
03/93	wound	vascular surgery	8	20020002020	III29	11
12/92	HCW	surgical ICU	9	20020002020	III29	11
12/92	wound	surgical ICU	10	20020002020	III29	11
12/92	wound	surgical ICU	11	00020002020	III29	11
01/93	IVC	surgical ICU	12	20020002020	III29	11
01/93	nose	surgical ICU	13	20020002020	III29	11
03/93	bile	surgical ICU	14	20020002020	III29	11
12/92	blood	general surgery	15	00020002000	III29	11
01/93	wound	general surgery	16	20020002020	III29	11
04/93	wound	general surgery	17	02020002020	III29	11
CONTROL GROUP 1						
08/92	nose	surgery	18	20020002020	III95	9
12/92	urine	plastic surgery	19	10022022220	E1	7
05/93	wound	dermatology	20	20002022200	Z74	9
11/86	sputum	cardiac surgery	21a/b	20022022220	E1	7
12/86	faeces	internal medicine	22	20022002220	E1	7
CONTROL GROUP 2[h]						
05/92	NA		23	20020002220	III29	10
01/92	NA		24	20020002021	III29	11
02/91	NA		25	20020002020	III29	6
04/91	NA		26	20020002020	III29	10
06/91	NA		27	20020002020	III29	ND[i]
06/91	NA		28	20020002220	III29	11
08/91	NA		29	20020002020	III29	11
11/91	NA		30	20020002220	III29	11

a ASP, air settling plate; IVC means intravascular catheter; NA, not available.
b a/ b strain typed in duplicate by PFGE.
c The values shown are for, from left to right, phosphomycin, oxacillin, tobramycin, co-trimoxazole, ciprofloxacin, erythromycin, clindamycin, vancomycin, rifampicin, fusidic acid and tetracycline. In the antibiogram, 0 indicates resistance, 2 indicates susceptible and 1 indicates intermediate susceptibility.
d Number repetitive units present in the protein A gene.

IS*431* type	Type determined by PCR primer species[e]: A	B	C	D	E	AP-PCR type[f]	PFGE type[f]	combined PFGE; AP-PCR type[g]	combined PFGE;AP-PCR; Protein-A;IS*431* type[g]
1	1'	1	1'	1	1	A3	A1/A1	1'	1'
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1'	1	1'	1	1	A3	A1/A1	1'	1'
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A3	1"	1"
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1	1	1	1	1	A1	A2	1	1
1	1'	1	1'	1	1	A3	A1	1'	1'
1	1	1	1	1	1	A1	A2	1	1
1	1	1'	1	1	1	A2	C	2	2
3	1'	2	1	1	2	B	D	3	3
2	2	3	2	1	3	C	E1	4	4
2	1'	4	3	1	1	D	F	5	5
5	2	3	2	1	3	C	E1/E2	4/4'	6/6'
5	2	3	2	1	3	C	E2	4'	6
1	3	1	1'	1	1	E	B2	6	7
1	4	1	4	1	1	F	A1	7	8
6	5	5	5	1	4	G	A1	8	9
6	6	6	6	2	5	H	A1	9	10
6	3	1	1'	1	1	E	A1	10	11
1	3	1'	1'	1	1	E'	G	11	12
ND	3	1	1'	1	1	E	A4	10	11
4	3	1	1'	1	1	E	B1	6	13

e A: primer 186, B: primer 188, C: primer 192, D: primer ERIC1 and E: primer ERIC2. Primes indicate one- or two-band differences.
f Subnumbering indicates subclonal relationships. Prime indicates one- or two band differences,
g Primes indicate subclonal variation.
h Control group 2 strains were derived from a collection of MRSA strains from various Dutch hospitals in the Dutch National Institute of Public Health and Environmental Protection (RIVM).
i ND, not determined.

AP-PCR and PFGE. AP-PCR based on a combination of several primer-dependant genocodes, revealed genetic homogeneity in the group of outbreak-related isolates all of which were type A (Table 2 and Figure 2). Three subtypes (A1 to A3) were identified within the outbreak-related isolates. As determined by PFGE analysis, all strains but one (isolate 17) were type A, although subclonal variation was observed (Table 2 and Figure 3). All strains identified as PFGE type A had a similarity index of more than 90%, as determined by UPGMA-analysis. Strain 17 was discriminated from the other outbreak-related isolates (similarity index of approximately 80%). Both AP-PCR and PFGE distinguished all control group 1 isolates from the outbreak-related isolates. Also, there was full agreement between PFGE and AP-PCR within this group of strains. Both AP-PCR and PFGE detected genetic heterogeneity among control group 2 strains. AP-PCR discriminated four types and PFGE detected three types, when subclonal variation is disregarded. However, some results of AP-PCR and PFGE typing were discordant. For instance, AP-PCR did not discriminate among isolates 23 and 27 to 30, whereas PFGE identified three different types (A, B, and G) within this group. On the other hand, PFGE did not discriminate among isolates 24 to 27 and 29 (PFGE type A), but AP-PCR identified four different types (E through H).
AP-PCR discriminated eight types and PFGE discriminated seven types in the total group of 30 strains. The combined results of AP-PCR and PFGE typing differentiated 11 types.
PCR mediated protein A typing. Protein A gene typing revealed 11 repeats in all outbreak-related isolates (Table 2 and Figure 4). In contrast, only seven or nine repeats were identified in isolates from control group 1. Four of eight isolates from control group 2 also contained 11 repeats.

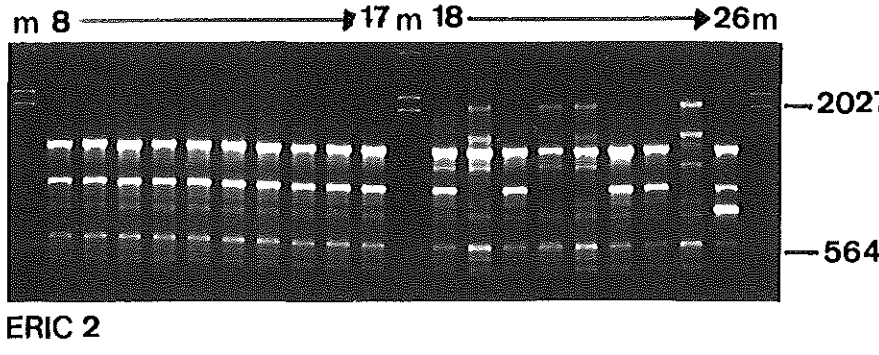

Figure 2: *Representative examples of the results of PCR mediated DNA fingerprinting using primer ERIC2. The isolate number is indicated above the lanes (in accordance with table 1). On the left, right and in the middle, molecular weight markers (marked "m"; Lambda DNA, cut with HindIII) are indicated in basepairs.*

IS*431* probing. Table 2 shows the results of IS*431* typing. All outbreak-related isolates and three of eight isolates from control group 2 were IS*431* type 1, whereas none of the isolates in control group 1 were of this type.
Combination of genotypic methods. Together the genotyping methods (AP-PCR, PFGE, Protein A and IS*431*) discriminated 13 different types among all isolates tested. All outbreak strains but one (the methicillin-susceptible isolate 17) were similar when subclonal variation was disregarded. Eleven different strains were identified among the 13 isolates from control group 1 and 2. The two control group 1 strains that could not be distinguished from each other (21 and 22) were isolated from patients who, within a 3-week period, were transferred to our hospital from the same foreign hospital.

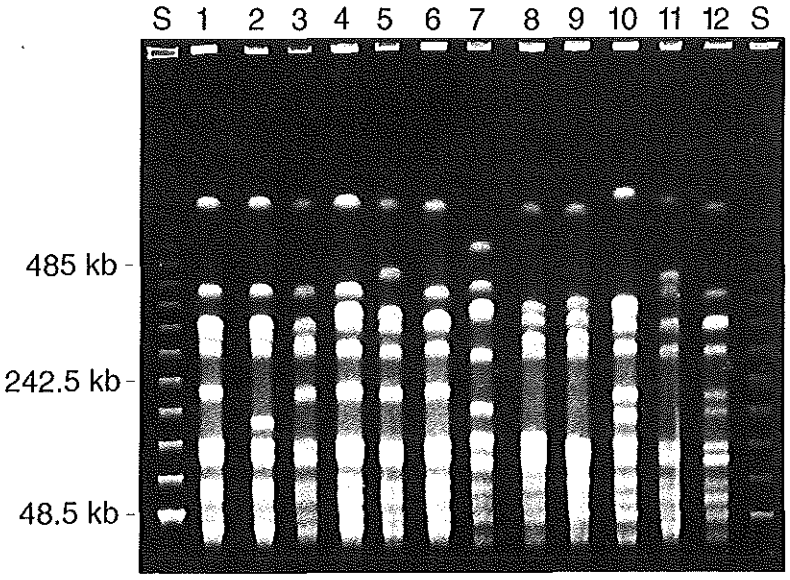

Figure 3: *PFGE mediated DNA typing of MRSA strains. DNA was digested with the restriction enzyme SmaI. Lanes marked S contain concatemeric forms of lambda DNA, differing in size from 48.5 to 485 kilobasepairs as indicated on the left. Lanes marked 1 to 12 contain DNA from the following strains, respectively: 1. strain 4, PFGE type A2; 2. strain 17, PFGE type C; 3. strain 10, PFGE type A3; 4. strain 5a, PFGE type A1; 5. strain 23, PFGE type B2; 6. strain 29, PFGE type A4; 7. strain 18, PFGE type D; 8. strain 19, PFGE type E1; 9. strain 21, PFGE type E2; 10. strain 20, PFGE type F; 11. strain 28, PFGE type G; 12 strain 30, PFGE type B1.*

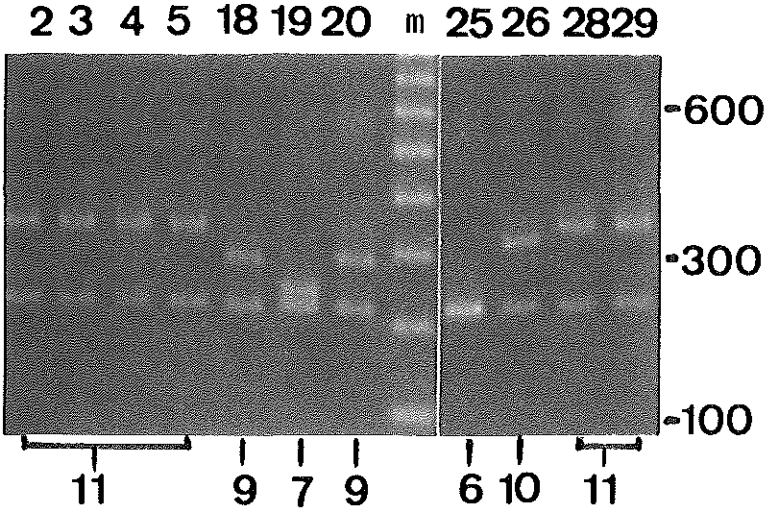

Figure 4: *Typing of protein A gene polymorphisms by PCR and RsaI RFLP analysis. Shown are representative examples of all types that were encountered during the analysis. Isolate numbers are displayed above the lanes. The lane marked "m" shows molecular weigth markers (Pharmacia 100 bp ladder). On the right the length of some of the markers is indicated in basepairs. Below the lanes the length of the variable fragment is indicated in repeat unit number.*

DISCUSSION

Despite the low incidence (<5%) of MRSA in Dutch hospitals, this organism regularly causes outbreaks.[33] In order to prevent outbreaks and decrease the endemic incidence of MRSA, we need to define the mechanisms by which this multiresistant microorganism is transmitted. Numerous investigators have used traditional epidemiological and molecular typing methods to identify risk factors for acquisition of MRSA, the reservoirs occupied by MRSA, and the means by which it is transmitted, yet we have much to learn about the epidemiology of this important pathogen.

We used several pheno- and genotyping methods to investigate an unusual explosive outbreak of MRSA in the University Hospital Rotterdam. The likely source of this outbreak was a dietary worker who prepared food for patients on the hematology unit. We identified this individual, who had no direct contact with patients, because we found the epidemic strain in a routine surveillance culture of a banana which the dietary work had pealed. A subsequent culture of the dietary worker's throat revealed that he carried the epidemic strain. In contrast, the index patient's surveillance cultures were negative for 3 weeks before she developed MRSA sepsis. To our knowledge, this is the first time investigators have implicated contaminated food in the transmission of MRSA. In general, ingestion of MRSA should not lead to subsequent infection because the gastric acid and the normal gastrointestinal flora should prevent this organism from colonizing the gastrointestinal tract. Moreover, the immune system usually eliminates transient invading microorganisms. The index patient, however, was severely immunocompromised, and she had taken both antacids, which would neutralize the gastric acid, and oral ciprofloxacin, which may have selected the ciprofloxacin-resistant outbreak strain. We postulate that the index patient ingested food contaminated by the outbreak strain, which then colonized the patient's gastrointestinal tract. Subsequently, while the patient was neutropenic the outbreak strain translocated through the gastrointestinal mucosa into the bloodstream, causing the patient's fatal infection.

A nurse who initially worked on the hematology unit probably transmitted the outbreak strain between wards when she was transferred to the vascular surgery unit. In the latter unit, the outbreak strain caused an explosive MRSA epidemic. By using stringent infection control measures, we terminated the outbreak within 6 months, and we eliminated the outbreak strain from our hospital. Although many infection control experts consider instituting an isolation facility outside of the hospital an extreme measure, we feel that in this particular outbreak it was better than other systems of isolation. First, because the outbreak strain was airborne and hence contaminated the environment extensively, we think the isolation unit enabled us to control this outbreak more quickly than we could have if the patients have been cared for within the hospital. Second, because all patients in the isolation facility had MRSA the patients were not kept in strict isolation. The patients were able to move about the ward as they desired, which may have enhanced their recovery. It should be remembered that in The Netherlands there is a national guideline for the control of MRSA, aimed at total elimination of MRSA and not confinement to a low endemic level.

The ratio of MRSA-infected to MRSA-colonized patients in this outbreak (78%) was much higher than that observed in previous outbreaks (up to 40%).[20] The increased frequency of infection and the high mortality rate (5 of 27; 18.5%) may have been related in part to the severity and nature of the patients' underlying diseases. The index patient was severely immunocompromised, and the patients on the vascular surgery unit had large, poorly healing wounds and many had prosthetic vascular grafts. In addition, we think the outbreak strain might have been more virulent than other MRSA strains. For methicillin-susceptible *S. aureus,* it has been shown that there are differences in virulence between strains.[36] Because

protein A modulates opsonization and chemotaxis,[35] the outbreak strain, which had more copies of the protein A gene than did many control isolates, might have evaded host defenses more readily than other strains.

We routinely evaluate the ability of all patients with MRSA to disperse the organism into the air and hence to contaminate the environment. Of the 20 patients with MRSA who were transferred to our hospital from foreign hospitals before the outbreak, none dispersed MRSA to the extent that we observed in this epidemic. Air-settling plates for 16 of those 20 patients were negative and for 4 patients contained <5 CFU per plate. In contrast, air settling plates in the rooms of several patients from this outbreak contained >100 CFU. Furthermore, despite the patients kept in strict isolation, air settling plates in the corridors outside of patients' rooms were also positive for the outbreak strain. We think that the positive air pressure in the patients' rooms carried MRSA from the contaminated rooms into the hallway. Although staff who carried the outbreak strain may have contaminated the settling plates in the corridors, we do not think that this was the likely mechanism. We made cultures from specimens from the staff twice weekly to identify those who acquired MRSA, and we tested all positive staff [16] to determine whether they dispersed the organism. We did not identify any staff members who dispersed MRSA during their tests.

Particular characteristics of the affected patients and the outbreak strain may have facilitated the profuse environmental contamination noted in this outbreak. Boyce *et al.* previously reported that patients who had MRSA in a wound or in their urine contaminated the environment more frequently than did patients with MRSA in the nares or sputum (34% versus 2% of the surfaces cultured were positive, respectively).[5] Therefore, our patients who had large, poorly healing wounds may have been more likely to disperse MRSA.

Other investigators have observed differences in the transmissibility of *S. aureus* strains.[8,10] Recently, Frenay *et al.* proposed that the number of repetitive elements in the spacer region of the staphylococcal protein A gene correlated with the ability of MRSA strains to cause outbreaks.[12] They argued that higher numbers of repeats enhanced the accessibility of the immunogloblin G binding region of protein A and thereby contributed to epidemic spread of the organism. Our outbreak strain had 11 copies of the protein A gene, which is within the epidemic range (more than 7) defined by Frenay *et al.*[12]

In summary, we hypothesize that patients who had wounds that were colonized or infected with the outbreak strain dispersed MRSA into the air. The airborne organisms subsequently contaminated many surfaces in the patients' rooms and corridors. HCWs who touched these contaminated surfaces and who did not subsequently wash their hands transmitted the epidemic strain to other patients. As in the study of Boyce *et al.*,[5] HCWs may also have contaminated their clothing and transmitted the outbreak strain in this manner.

Because the incidence of MRSA in The Netherlands is extremely low, isolates obtained during outbreaks are likely to be clonally related. Therefore, we evaluated isolates from our outbreak by several molecular typing methods, and we compared the results of the different methods. AP-PCR and PFGE most clearly discriminated outbreak-associated isolates from control isolates. In our study, PFGE identified more subclones than did PCR. Saulnier *et al.* noted similar results,[27] but further analysis determined that the discriminatory abilities of PFGE and PCR depended, respectively, on the number of restriction enzymes and the number of primers used.[32] Furthermore, as demonstrated in Table 2, some PCR primers detected more variability than did others; four primers identified five or more types, whereas primer ERIC1 identified only two types. To date, no one has determined which primers and how many primers should be used to evaluate MRSA isolates.

At present, we do not know why MRSA isolates obtained during the course of an outbreak had minor genomic differences. However, Van Belkum *et al.* noted similar variability among

Legionella pneumophila.[31] Perhaps this variability reflects the intrinsic genetic flexibility of the microbes or their ability to adapt to a host.

Both PFGE and PCR were more discriminatory than protein A typing or IS*431* typing. We identified the most strain variability when we combined the results of these four genotypic typing methods. Using the combined results, we considered all but one of the outbreak-related isolates (isolate 17) to be the same strain. We did not identify the outbreak strain among the control isolates.

Only PFGE and methicillin susceptibility discriminated isolate 17 from the other outbreak isolates. Isolate 17 possessed the *MecA* gene (documented by PCR) but did not express methicillin-resistance. At present we do not know the mechanism by which this isolate remains susceptible to methicillin.

None of the typing methods distinguished between two isolates in control group 1 (isolates 21 and 22). We subsequently learned that these isolates were obtained from two patients who, within a short time period, were transferred to our hospital from the same foreign hospital. Hence, the patients may have acquired the same strain while they were in the other hospital.

In summary, our MRSA outbreak displayed several unusual features, including transmission of the outbreak strain by food and by air, and a very high infection-to-colonization ratio. We think the patients' wounds and particular characteristics of the outbreak MRSA strain allowed that strain to spread explosively on our vascular surgery unit. Furthermore, we think the isolation unit helped us terminate the outbreak expeditiously. Finally, PFGE and AP-PCR discriminated the outbreak-related isolates from control isolates, and protein A typing suggested that the outbreak strain might be more transmissible and virulent than other MRSA isolates.

Acknowledgements: The authors gratefully acknowledge Marly Simons for performing the protein A typing assays, the hospital infection control nurses, Marie-Jose Manders and Anita Tomaello, for supporting the epidemiological investigation, and the medical and nursing staff of the departments of hematology and surgery for coöporating with us.

REFERENCES

1 **Archer GL, Niemeyer DM, Thanassi JA and Pucci MJ.** Dissemination among staphylococci of DNA sequences associated with methicillin resistance. *Antimicr Agents Chemother* 1994;**38**:447-54.

2 **Barberis-Maino L, Berger-Bachi B, Weber H, Beck WD and Kayser FH.** IS431, a staphylococcal insertion sequence-like element related to IS26 from *Proteus vulgaris*. *Gene* 1987;**59**:107-13.

3 **Bauer AW, Kirby WM, Sherris JC and Turck M.** Antibiotic susceptibility testing by a standardised single disk method. *Am J Clin Pathol* 1966;**45**:493-6.

4 **Bialkowska-Hobranzka H, Harry V, Jaskot D, and Hammerberg D.** Typing of coagulase-negative staphylococci by southern hybridisation of chromosomal DNA fingerprints using ribosomal RNA probe. *Eur J Clin Microbiol Inf Dis* 1990;**9**:588-94.

5 **Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van Dillen PME and van der Noordaa J.** Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990;**28**:495-503.

6 **Boyce JM, Potter-Bynoe G, Chenevert C and Hughes NL.** Contamination of environmental surfaces with methicillin-resistant *Staphylococcus aureus* (MRSA): Infection control implications. *Program and abstracts of the fourth annual meeting of the society of hospital epidemiology of America.* New Orleans, LA, 1994. Abstract 14, page 22.

7 **Carbs-Nurit MJ, Christophe B, Brooke S, Gomby A, Bouziges N and Ramenez M.** DNA polymorphisms in methicillin-susceptible and methicillin-resistant strains of *S aureus*. *J Clin Microbiol* 1992;**30**:2092-6.

8 **Casewell MW.** Epidemiology and control of the 'modern' methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 1986;**7**(suppl. A):1-11.

9 **Dice LR** Measures of the amount of ecological association between species. *Ecology* 1945;**26**:379-92.

10 **Duckworth G.** Revised guidelines for the control of epidemic methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 1990;**16**:351-77.

11 **Felsenstein J.** PHYLIP-Phylogeny interference package (Version 3.2). *Cladistics* 1989;**5**:164-6.

12 **Frenay HM, Theelen JPG, Schouls LM, Vandenbroucke-Grauls CMJE, Verhoef J, van Leeuwen WJ and Mooi FR.** Discrimination of epidemic and non-epidemic strains of methicillin resistant *Staphylococcus aureus* on the basis of protein A gene polymorphisms. *J Clin Microbiol* 1994;**32**:846-7.

13 **Goering RV and Winters MA.** Rapid method for epidemiological evaluation of gram positive cocci by field inversion gel electrophoresis. *J Clin Microbiol* 1992;**30**:577-80.

14 **Goh SH, Byrne SK, Zhang JL and Chow AW.** Molecular typing of *S aureus* on the basis of coagulase gene polymorphisms. *J Clin Microbiol* 1992;**30**:1642-5.

15 **Hermans PWM, van Soolingen D, Bik EM, de Haas PEW, Dale JW and van Embden JDA.** Insertion element IS986 from *Mycobacterium tuberculosis*: a useful tool for diagnosis and epidemiology of tuberculosis. *J Clin Microbiol* 1990;**28**:2051-8.

16 **Huysmans-Evers AGM.** Results of routine testing for the detection of dispersal of *Staphylococcus aureus*. *Arch Chir Neerlandicum* 1978;**30**;141-50.

17 **Kreiswirth B, Kornblum J, Arbeit RD, Eisner W, Maslow JN, McGeer A, Low DE and Novick RP.** Evidence for a clonal origin of methicillin-resistance in *Staphylococcus aureus*. *Science* 1993;**259**:227-30.

18 **Leeuwen WJ and Rost JA.** Additional set of typing phages of *Staphylococcus aureus* strains of human strains not typable with the international basic set of phages. *Zentrall Bl Bacteriol* 1976;**suppl 5**: 1013-19.

19 **Mulligan ME, Kwak RYY, Citron DM, John JF jr. and Smith PB.** Immunoblots, antimicrobial resistance and bacteriophage typing of oxacillin resistant *S. aureus*. *J Clin Microbiol* 1988;**26**:2395-401.

20 **Mulligan ME, Murray-Leisure KA, Ribner BS, Standiford HC, John JF, Corvick JA, Kauffman CA, and Yu VL.** Methicillin-resistant *Staphylococcus aureus*: A consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. *Am J Med* 1993;**94**:313-28.

21 **Musser JM and Kapur V.** Clonal analysis of methicillin resistant *Staphylococcus aureus* strains from intercontinental sources: association of the mec gene with divergent phylogenetic lineages implies dissemination by horizontal transfer and recombination. *J Clin Microbiol* 1992;**30**:2058-63.

22 **National Committee for Clinical Laboratory Standards** (1990). Performance standards for antimicrobial disk susceptibility tests. 4th Edition Approved Standard, M2-A4, vol. 10, National Committee for Clinical Laboratory Standards, Villanowa, Pa.

23 **Parker MT** Page typing of *Staphylococcus aureus*. In: *Methods in microbiology*, Vol 11, 1978. Academic Press. London and New York.

24 **Pfaller MA, Hollis RJ and Sader HS.** PFGE Analysis of chromosomal restriction fragments. In: *Clinical Microbiology Handbook (Ed. H.D. Isenberg). Suppl. I, American Society for Microbiology*, Washington DC,1994 section 10.5.b.

25 **Saiki RK, Gelfland DH, Stoffer S, Scharff SJ, Higuchi R, Horn GR, Mullis HB and Ehrlich HA.** Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;**239**:487-91.

26 **Sambrook J, Fritsch EF and Manialis T.** Molecular cloning: *A laboratory manual*, 2nd Ed. 1989. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

27 **Saulnier P, Bourneix C, Prevost G and Andremont A.** Random amplified polymorphic DNA assay is less discriminant than pulsed field electrophoresis for typing strains of methicillin resistant *Staphylococcus aureus*. *J Clin Microbiol* 1993;**31**:982-5.

28 **Struelens MJ, Deplano A, Godard C, Maes N and Serruys E.** Epidemiological typing and delineation of genetic relatedness of MRSA by macrorestriction analyses of genomic DNA by using PFGE. *J Clin Microbiol* 1992;**30**:2599-605.

29 **Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S, Goering R, Hancock G, Huebert G, Hill B, Hollis R, Jarvis WJ, Kreiswirth B, Eisner W, Maslow J, McDougal LK, Miller JM, Mulligan M and Pfaller MA.** Comparison of traditional and molecular methods for typing isolates of *Staphylococcus aureus*. *J Clin Microbiol* 1994;**32**:407-15.

30 **Van Belkum A, Bax R, Peerbooms P, Goessens WHF, van Leeuwen N and Quint W.** Comparison of phage typing and DNA fingerprinting by PCR for discriminating methicillin resistant *S. aureus* strains. *J Clin Microbiol* 1993;**31**:798-803.

31 **Van Belkum A, Struelens M and Quint WGV.** Typing of *Legionella pneumophila* strains by polymerase chain reaction fingerprinting. *J. Clin. Microbiol.* 1993;**31**:2198-200.

32 **Van Belkum A, Bax R and Prevost G.** Comparison of four genotyping assays for epidemiological study of methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis.* 1994;**13**:420-4.

33 **Vandenbroucke-Grauls CM, Frenay HM, van Klingeren B, Savelkoul TF and Verhoef J.** Control of epidemic methicillin resistant *Staphylococcus aureus* in a Dutch University Hospital. *Eur J Clin Microbiol Inf Dis* 1991;**10**:6-11.

34 **Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT and Braveny I.** Methicillin resistant *Staphylococcus aureus* in Europe. *Eur J Clin Microbiol Infect. Dis.* 1994;**13**:50-5.

35 **Waldvogel F.** *Staphylococcus aureus* (including toxic shock syndrome). In: Mandell G.L., Gordon Douglass R, Bennett J. E. Eds. *Principles and Practice of Infectious Diseases, Churchill Livingstone*, 3rd edn. 1990;1489-510.

36 **Williams REO, Blowers R, Garrod LP, and Shooter RA** *Hospital Infection; Causes and Prevention.* Lloyd-Luke (medical books) LTD, 2nd edn. 1966.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The purpose of the studies presented in this thesis was to gain more insight into the current epidemiology of *S. aureus* in the hospital setting and to develop strategies to prevent these infections.

In Chapter 1 surgical wound infections in cardio-thoracic surgery are studied. Part 1 shows the results of an 18-month prospective surveillance of postoperative infections in the department of thoracic surgery of the University Hospital Rotterdam-Dijkzigt, The Netherlands. Out of 983 patients, 194 (19.7%) developed one or more postoperative infections. A total of 268 postoperative infections were diagnosed, resulting in an incidence of 2.0 infections per 100 days of postoperative stay. The mean postoperative length of stay of the 194 patients with postoperative infections was two weeks longer than that of patients without postoperative infections. Deep surgical wound infections were associated with the longest prolongation of the median postoperative length of stay in the hospital (one month longer). Incisional surgical wound infections were also associated with a significant prolongation of stay (median 10 days longer). *S. aureus* was the most important pathogen associated with surgical wound infections. Phage typing of 29 strains causing surgical wound infections showed two identical pairs. Thus, only a small minority of wound infections with *S. aureus* could be explained by cross-infection. It was subsequently hypothesized that endogenous strains of *S. aureus*, i.e. those already present on the patient at time of admission, play a major role in the development of wound infection. To evaluate the relative importance of nasal carriage of *S. aureus* as a risk factor for the development of wound infection at the sternotomy site after cardiac surgery, a case control study was performed (part 2). The study population consisted of 1,980 consecutive patients. Cases were all patients who developed a sternal wound infection from which *S. aureus* was cultured. Forty cases were identified, and 120 controls were selected. Preoperative nasal carriage of *S. aureus*, insulin dependent diabetes mellitus and younger age were identified as significant riskfactors. The crude odds ratio of nasal carriage was 9.6 (95% confidence interval: 3.9-23.7). The median postoperative length of stay in cases was 30 days longer than in controls. Also, mortality was significantly higher among cases than controls (10.0% and 0.8%, respectively). The conclusion was that nasal carriage of *S. aureus* is a major risk factor for the development of sternal wound infections after cardiac surgery. These findings suggested that perioperative elimination of nasal carriage could reduce the incidence of surgical wound infections. In part 3 this hypothesis was tested, using mupirocin nasal ointment to eliminate nasal carriage in patients undergoing cardiothoracic surgery. In 928 patients without mupirocin the surgical wound infection rate was 7.3%. After the introduction of mupirocin the rate dropped to 2.8% (n=868, P<0.0001). Mupirocin prophylaxis was wel tolerated and showed no side-effects. Development of resistance was not observed. However, the interpretation of these promising results should be made cautiously, because a historic control group was used. General application of this new preventive strategy should await confirmation of its efficacy in a double-blind, randomized, placebo-controlled trial. The costs and benefits of perioperative nasal mupirocin in cardio-thoracic surgery were evaluated in part 4. Using an appropriateness evaluation protocol, the savings in postoperative length of stay corresponded with $115 per patient and the savings in procedure related costs were $225 per patient. Since the costs of mupirocin were only $11 per patient, the net effect was financially highly profitable. The estimated savings were $329 per patient undergoing cardiothoracic surgery.

Another group of patients who are at high risk for infection with *S. aureus*, are those treated with hemodialysis because of renal failure. In this group of patients extremely high carriage

rates are observed and most *S. aureus* infections are endogenous. The effect of elimination of nasal carriage using mupirocin is described in Chapter 2. Of the 226 hemodialysis patients during the study period, 172 were evaluable. Sixty-seven (39%) were identified as nasal carriers. Immediately after the initial treatment, 66 (98.5%) of them had negative nasal cultures. After 3 months and 6 months, 63 (94%) and 61 (91%) of the treated carriers still had negative cultures, respectively. To study the effect of elimination of nasal carriage on the incidence of *S. aureus* infections, the rate of bacteremia in the 226 patients in the study group was compared with this rate in a historic control group of 273 patients. In the study group four episodes of *S. aureus* bacteremia occurred on a total of 100.3 years on hemodialysis. In the control group 25 episodes occurred on 100.0 years ($P<0.001$). Development of resistance and adverse effects were not observed. Therefore, it was concluded that mupirocin nasal ointment effectively eliminated nasal carriage of *S. aureus* in patients on hemodialysis, and, thereby, significantly reduced the incidence of bacteremia. Since *S. aureus* has been recognized as an important pathogen for human disease for more than a century, the literature available on its epidemiology is extensive. In Chapter 3 the literature is reviewed with respect to the global epidemiology of methicillin-resistant *S. aureus* (MRSA) in part 1 and the epidemiology of nasal carriage in part 2. In part 1 it is concluded that the incidence of MRSA as a nosocomial pathogen is rapidly increasing. In many countries the incidence of MRSA now equals or exceeds the incidence of methicillin-susceptible isolates. Genetic typing studies of MRSA strains consisting of isolates from all over the world which were collected from 1961 untill the early 1990's, showed that MRSA strains have a high level of genetic relatedness. The gene encoding for methicillin resistance, called the *Mec*-a gene, is not easily acquired by *S. aureus*. One studied concluded that this may have happened only once. Therefore, clonal dissemination together with horizontal transfer and recombination are the most likely explanations for the current pandemic of MRSA. The future with respect to MRSA is to be feared, because there is only one antimicrobial agent left, i.e. vancomycin, for which *S. aureus* is uniformally susceptible. However, in experimental studies it has been shown that vancomycin resistance can be transferred from vancomycin-resistant enterococci into MRSA. For such a microorganism no effective treatment would be available, and consequently the morbidity and mortality of infection with it would be enormous. Further insight into the epidemiology of MRSA and into the effectiveness of various control measures should help to keep this problem under control. In part 2 a review of the literature concerning nasal carriage of *S. aureus* is given. It is concluded that nasal carriage of *S. aureus* is a major risk factor for infection in several groups of patients. These include patients on hemodialysis. on continuous ambulatory peritoneal dialysis (CAPD), on surgery, with intravenous devices and with HIV. All these groups will increase in number in the near future, probably increasing the number of associated infections. In patients on hemodialysis, CAPD and surgery it has been shown that elimination of nasal carriage reduces the infection rates. In order to develop an optimal strategy for prevention it is important to gain insight into the underlying mechanisms of nasal carriage and into the epidemiology of *S. aureus* infection at the molecular level.
Therefore, typing methods are of utmost importance. The recent developments in molecular microbiology have resulted in several promising new techniques which are studied in Chapter 4. One of these techniques, arbitrarily primed polymerase chain reaction (AP-PCR), was evaluated for its reproducability and discriminatory power (part 1). A well defined collection consisting of 59 *S. aureus* and 1 *S. intermedius* isolates was used. Seven centers collaborated and used a standardized amplification protocol, template DNA isolated and distributed from our istitute, as was a set of three primers. Between the seven centers a

significant variation in discriminatory power of their AP-PCR procedure was observed. However, the clustering of epidemiologically related strains was correct in all centers. AP-PCR was found to be more discriminative than the more traditional typing strategies. Although the interlaboratory reproducibility needs improvement, it was concluded that AP-PCR was well suited for epidemiological investigations of *S. aureus*. In part 2 an outbreak of methicillin-resistant *S. aureus* (MRSA) in the University Hospital Rotterdam was analyzed by different typing methods. In this outbreak several interesting observations were made. The outbreak was probably caused by the contamination of food by a health care worker on the department of hematology. The strain subsequently spread to the department of surgery. Here an explosive outbreak occurred in large part caused by airborne transmission. The institution of a special MRSA-unit outside the hospital proved to be an effective measure to control the outbreak. The high morbidity and mortality of patients colonized with the outbreak strains were remarkable. Of the 27 patients colonized, 21 (77.8%) developed infections and five of them died. In three cases death was clearly caused by the MRSA infection. Analysis of the outbreak strains and collection of unrelated MRSA revealed that the outbreak strains were all identical. Furthermore, the discriminatory power of genotypic typing methods was superior to phenotypic methods. Optimal results were achieved by combination of several genotypic methods.
In conclusion, this thesis has focussed on the role of nasal carriage as a risk factor for infection in specific groups of patients. It was also shown that elimination of nasal carriage in high risk groups reduces the incidence of infection. This new strategy gives new perspectives in our ecological interactions with this species. In addition to endogenous infection it has been noted that cross-infection is an important mechanism for this opportunistic pathogen as well. To keep pace with the ongoing adaptation of *S. aureus*, new strategies have to be developed. The role of nasal carriage appears to be crucial both in the development of self-infection and in the spread of the organism. More insight into the underlying mechanisms of the carrier-state should lead to new strategies for treatment and prevention which are essential to control staphylococcal infections in the future.

SAMENVATTING EN CONCLUSIES

Het doel van de onderzoeken beschreven in dit proefschrift was om meer inzicht te verkrijgen in de epidemiologie van *Staphylococcus aureus* infecties in het ziekenhuis en om nieuwe strategiën te ontwikkelen om dergelijke infecties te voorkomen.
In Hoofdstuk 1 worden chirurgische wondinfecties in de cardio-pulmonale chirurgie bestudeerd. Deel 1 toont de resultaten van een prospectieve surveillance gedurende 18 maanden in de afdeling cardio-pulmonale chirurgie van het Academisch Ziekenhuis Rotterdam-Dijkzigt. Van de 983 patienten ontwikkelden 194 (19.7%) een of meer postoperatieve infecties. In totaal werden 268 postoperatieve infecties vastgesteld, hetgeen resulteert in een incidentie van 2.0 infecties per 100 dagen post-operatieve opnameduur. De gemiddelde postoperatieve opnameduur van de 194 patienten met postoperatieve infecties was 2 weken langer dan die bij patienten zonder infecties. Diepe chirurgische wondinfecties gingen gepaard met de langste opnameduur verlenging (mediaan: 30 dagen). Oppervlakkige chirurgische wondinfecties waren ook geassocieerd met een significante verlenging van de opnameduur (mediaan: 10 dagen). *S. aureus* was het belangrijkste pathogene micro-organisme bij wondinfecties. Faagtypering van de 29 stammen die bij chirurgische wondinfecties waren geïsoleerd toonde 2 identieke paren. De conclusie was dat slechts een kleine minderheid van de wondinfecties met *S. aureus* kon worden toegeschreven aan een kruisinfectie. Dit leidde tot de hypothese dat het merendeel van de infecties veroorzaakt

werd door endogene *S. aureus* stammen. Hiermee wordt bedoeld die stammen die reeds preoperatief deel uitmaken van de patient zijn of haar eigen flora.
Om het relatieve belang van neusdragerschap van *S. aureus* als een risicofactor voor wondinfecties met dit microorganisme na sternotomie te bepalen werd een patient-controle onderzoek verricht, zoals beschreven in deel 2. De studiegroep bestond uit 1.980 opeenvolgende patienten die hartchirurgische ingrepen ondergingen. De defintie van een patient was dat deze een sternotomie wondinfectie ontwikkelde waaruit *S. aureus* werd gekweekt. Er werden 40 patienten geïdentificeerd en 120 controles geselecteerd. Preoperatief neusdragerschap met *S. aureus*, insuline afhankelijke diabetes mellitus en jonge leeftijd werden als onafhankelijke, significante risicofactoren geïdentificeerd. De ongecorrigeerde odds ratio van neusdragerschap was 9.6 (95% betrouwbarheidsinterval: 3.9-23.7). De mediane postoperatieve opnameduur van patienten was 30 dagen langer dan die van controles. Ook de sterfte was significant hoger bij patienten (10%) in vergelijking met de controles (0.8%). De conclusie was dat neusdragerschap met *S. aureus* een belangrijke risicofactor is voor het ontwikkelen van een sternotomie wondinfectie na hartchirurgie. Deze bevindingen maken het aannemelijk dat perioperatieve eliminatie van neusdragerschap de incidentie van wondinfecties kan verminderen. In deel 3 werd deze hypothese getoetst, door mupirocine neuszalf perioperatief toe te dienen bij patienten die cardio-pulmonale chirurgie ondergingen. In 928 patienten zonder mupirocine was de incidentie van chirurgische wondinfecties 7.3%. Na de introductie van mupirocine daalde deze incidentie tot 2.8% (n=868, P<0.0001). Mupirocine prophylaxe werd goed verdragen en er waren geen belangrijke bijwerkingen. Resistentie tegen mupirocine werd niet waargenomen. Opgemerkt dient te worden dat zorgvuldigheid geboden is bij de interpretatie van deze bevindingen omdat er een historische controle groep werd gebruikt. Algemene toepassing van deze nieuwe preventieve maatregel kan pas aanbevolen worden na bevestiging in een dubbel-blinde, gerandomiseerde, placebo-gecontroleerde studie. De kosten en de baten van mupirocine in cardio-pulmonale chirurgie werden geëvalueerd in deel 4. Met behulp van een "appropriateness evaluation protocol" werden de besparingen ten gevolge van minder postoperatieve opnameduur bepaald op NLG 207 per patient en de besparingen ten gevolge van verrichting gerelateerde kosten op NLG 405 per patient. Omdat de kosten van mupirocine slechts NLG 19.8 per patient waren, was de eindbalans in hoge mate kosteneffectief met een besparing van NLG 592 per patient die cardio-pulmonale chirurgie ondergaat. Nadere analyse toonde dat variaties in de kosten van mupirocine, in de incidentie van chirurgische wondinfecties en in de effectiviteit van mupirocine nauwelijks van invloed waren op de kosten-effectiviteits verhouding. Alleen variaties in de hoogte van de meerkosten van wondinfecties was van invloed.
Een andere groep patienten die een hoog risico hebben op infecties met *S. aureus* zijn patienten die vanwege terminale nierinsufficientie met hemodialyse worden behandeld. In deze groep is ook de incidentie van dragerschap hoger dan normaal en worden de meeste infecties veroorzaakt door endogene *S. aureus* stammen. Het effect van eliminatie van neusdragerschap met behulp van mupirocine op de incidentie van *S. aureus* infecties in deze groep wordt beschreven in hoofdstuk 2. Van de 226 hemodialyse patienten in de studiegroep waren er 172 evalueerbaar; van deze laatste groep was 67 (39%) neusdrager. Direkt na de behandeling van de dragers waren de neuskweken van 66 patienten (98.5%) negatief geworden. Na 3 en 6 maanden waren respectievelijk 63 (94%) en 61 (91%) nog steeds niet gerekoloniseerd. Om het effect van de eliminatie van neusdragerschap op het voorkomen van bacteremiën met *S. aureus* te bestuderen werden de incidenties hiervan in de mupirocine behandelde groep vergeleken met die in een historische controle groep (n=273).

In de behandelde groep werden 4 episoden van bacteremiën met *S. aureus* waargenomen op een totale expositieduur van 100.3 hemodialyse jaren. In de controle groep waren 25 episoden in 100.0 hemodialyse jaren ($P<0.001$). Ontwikkeling van resistentie tegen mupirocine en bijwerkingen werden niet waargenomen. De conclusies waren dat mupirocine neuszalf effectief het neusdragerschap met *S. aureus* elimineerde en op die manier de incidentie van bacteremiën significant verminderde.
Aangezien *S. aureus* reeds meer dan een eeuw herkend wordt als een belangrijk pathogeen voor de mens, is de literatuur over dit microorganisme zeer uitgebreid. In hoofdstuk 3 wordt deze samengevat met betrekking tot de mondiale epidemiologie van methicilline resistente *S. aureus* (MRSA) in deel 1 en de epidemiologie van neusdragerschap van *S. aureus* in deel 2. In deel 1 wordt allereerst vastgesteld dat de incidentie van MRSA als een verwekker van ziekenhuisinfecties zeer snel toeneemt. In vele landen is de isolatie-frequentie van MRSA nu gelijk aan of groter dan die van gevoelige *S. aureus* stammen. Met behulp van genetische typeringstechnieken zijn grote aantallen MRSA stammen die wereldwijd verzameld zijn tussen 1961 en 1990 onderzocht. Hierbij werd een hoge mate van verwantschap gevonden. Het gen dat codeert voor methicilline resistentie, het *Mec*-a gen genaamd, wordt kennelijk niet makkelijk in het genoom van *S. aureus* opgenomen. Een studie concludeerde dat dit mogelijk slechts één maal gebeurd is. De huidige pandemie van MRSA lijkt het gevolg van clonale verspreiding in combinatie met sporadische horizontale overdracht naar een nieuwe cloon. Met betrekking tot de toekomst van MRSA moet het ergste worden gevreesd, omdat er nog maar een antibioticum is waarvoor alle stammen gevoelig zijn, namelijk vancomycine. In experimentele setting is echter aangetoond dat vancomycine resistentie kan worden overgedragen van vancomycine-resistente enterococcen op MRSA. Voor een dergelijk microorganisme zou op dit moment geen effectieve behandelingsmogelijkheid meer zijn, hetgeen zeer ernstige gevolgen met betrekking tot morbiditeit en mortaliteit zal hebben indien het zich verspreid. Een beter inzicht in de epidemiologie van MRSA en in de waarde van verschillende controlerende maatregelen zouden dit probleem beheersbaar moeten houden. In deel 2 wordt de literatuur over neusdragerschap van *S. aureus* weergegeven. De conclusie is dat neusdragerschap van *S. aureus* een belangrijke risicofactor is voor infecties in verschillende groepen patienten. Deze omvatten patienten met hemodialyse, met continue ambulante peritoneaal dialyse (CAPD), chirurgische patienten, met intravasale lijnen en met HIV. Al deze groepen zullen in de nabije toekomst in omvang toenemen, hetgeen het aantal daarmee geassocieerde infecties zal verhogen. Bij patienten die hemodialyse, CAPD of operatieve ingrepen ondergaan is gevonden dat eliminatie van neusdragerschap de kans op infectie sterk vermindert. Dit kan een uitermate belangrijke preventieve maatregel blijken. Om de optimale strategiën voor behandeling en preventie te ontwikkelen is het van groot belang om op moleculair niveau inzicht te verkrijgen in de onderliggende mechanismen van neusdragerschap en in de epidemiologie van *S. aureus* infecties.
Het inzicht in de epidemiologie van *S. aureus* infecties vereist goede typeringstechnieken. Recent ontwikkelingen in de moleculaire biologie hebben een aantal nieuwe technieken opgeleverd, welke in hoofdstuk 4 werden bestudeerd. In deel 1 werd een van de nieuwe technieken, namelijk arbitrarily primed polymerase chain reaction (AP-PCR), op zijn reproduceerbaarheid en onderscheidend vermogen getest, in verschillende centra. Een goed gedefinieerde collectie bestaande uit 59 *S. aureus* en 1 *Staphylococcus intermedius* werd hiertoe gebruikt. De 7 aan dit onderzoek deelnemende centra gebruikten een gestandaardiseerd amplificatie protocol, het DNA werd geïsoleerd in het Academisch Ziekenhuis Rotterdam, evenals een set van 3 verschillende primers. Tussen de verschillende centra werden grote verschillen in discriminerend vermogen van de door hun uitgevoerde

AP-PCR procedure waargenomen. Toch was de herkenning van epidemiologische relaties tussen de stammen in alle centra correct. AP-PCR discrimineerde beter dan de meer traditionele typeringstechnieken. Hoewel de reproduceerheid tussen de verschillende laboratoria voor verbetering vatbaar is, was de conclusie dat deze techniek goed bruikbaar is om epidemiologische studies van *S. aureus* te verrichten. In deel 2 wordt een epidemie met MRSA in het Academisch Ziekenhuis Rotterdam geanalyseerd met behulp van verschillende typeringstechnieken. De epidemie werd waarschijnlijk veroorzaakt door de besmetting van voedsel door een personeelslid op de afdeling hematologie. De stam werd daarna verspreid naar de afdeling heelkunde, waar een heftige epidemie ontstond ten gevolge van verspreiding via de lucht. Het instellen van een speciale MRSA-unit buiten het ziekenhuis bleek een effectieve maatregel om de epidemie onder controle te brengen. De hoge morbiditeit en sterfte onder patiënten die gekoloniseerd werden met de epidemische MRSA-stam was opmerkelijk. Van de 27 gekoloniseerde patienten ontwikkelden 21 (77.8%) een infectie en 5 overleden. In 3 gevallen kon het overlijden direct in verband worden gebracht met de MRSA-infectie. Analyse van de stammen geïsoleerd tijdens de epidemie en een collectie van niet-gerelateerde MRSA stammen toonde aan dat de stammen uit de epidemie allen identiek waren. Verder was het onderscheidende vermogen van de genotypische typeringsmethode superieur aan die van de phenotypische methoden. Het beste resultaat werd verkregen als de gegevens van meerdere genotypische methoden gecombineerd werden.

Concluderend: Dit proefschrift beschrijft onderzoek dat zich gericht heeft op de risicofactor neusdragerschap van *S. aureus*. In verschillende belangrijke patiënten groepen blijkt neusdragerschap geassocieerd te zijn met een sterk verhoogde kans op infectie. Eliminatie van neusdragerschap in risico-groepen vermindert de kans op *S. aureus* infecties aanzienlijk. Deze preventieve maatregel biedt nieuwe perspectieven in onze omgang met dit belangrijke microorganisme. Om de voortdurende evolutie van *S. aureus* bij te houden dienen meer van dergelijke nieuwe preventieve maatregelen te worden ontwikkeld. Neusdragerschap lijkt een sleutelrol te vervullen in het ontstaan van endogene infecties en in de verspreiding van het microorganisme. Een beter inzicht in de onderliggende mechanismen kan resulteren in nieuwe preventieve methoden die essentieel zijn om *S. aureus* infecties in de toekomst beheersbaar te houden.

PUBLICATIONS

PUBLICATIONS

1 **Wagenvoort JHT, Kluytmans JAJW, Veenendaal HR, Niemantsverdriet PCJ and Groothuis DG.** Screening for Legionnaires' disease by routine sputum culture and its serology. *Ant v Leeuwenhoek* 1985;**51**:520-1.

2 **Wagenvoort JHT, van Rijsoort-Vos JH, Kluytmans JAJW, Kieskamp MJ, Stolz E and Michel MF.** Trends in gonococcal resistance to antibiotics in Rotterdam, the Netherlands, 1975-86. *Genitourin Med* 1987;**63**:244-5.

3 **Kluytmans JAJW, van der Willigen AH, van Heyst BYM, van der Meyden WI, Stolz E and Wagenvoort JHT.** Evaluation of an enzyme immunoassay for detection of *Chlamydia trachomatis* in urogenital specimens. *Int J Sex Transm Dis & AIDS* 1990;**1**.49-52.

4 **Kluytmans JAJW, Niesters HGM, Mouton JW, Quint WGV, IJpelaar JAJ, van Rijsoort-Vos JH, Habbema L, Stolz E, Michel MF, and Wagenvoort JHT.** Performance of a nonisotopic DNA probe for detection of *Chlamydia trachomatis* in urogenital specimens. *J Clin Microbiol* 1991;**29**:2685-9.

5 **Kluytmans JAJW.** Infectieregistratie op thoraxchirurgie. *Ned Tijdschr Geneeskd* 1991;**135**:1471

6 **Dijkshoorn-de Bruin L and Kluytmans JAJW.** Epidemiologie en preventie van Ziekenhuisinfecties. In: Ontwikkelingen in de behandeling van infectieziekten. Bakker-Woudenberg I, Horrevorts A. (Eds). ICG printing, Dordrecht, 1991:104-13.

7 **Kluytmans JAJW, Mouton JW, Manders MAAJ, Maat APWM and Wagenvoort JHT.** Infectieregistratie en -preventie op een thoraxchirurgische afdeling. *Ned Tijdschr Geneeskd* 1992;**136**:1840.

8 **Kluytmans JAJW, Goessens WHF, Mouton JW, van Rijsoort-Vos JH, Niesters HGM, Quint WGV, Habbema L, Stolz E and Wagenvoort JHT.** Evaluation of Clearview and Magic Lite tests, PCR and cell culture for detection of *Chlamydia trachomatis* in urogenital specimens. *J Clin Microbiol* 1993;**12**:3204-10.

9 **Kluytmans JAJW, Goessens WHF, van Rijsoort-Vos JH, Niesters HGM and Stolz E.** Improved performance of PACE 2 with modified collection system in combination with Probe Competion Assay for detection of *Chlamydia trachomatis* in urethral specimens from males. *J Clin Microbiol* 1994;**32**:568-70.

10 **Kluytmans JAJW.** 1995. The clonal spread of resistant organisms: Methicillin resistant *Staphylococcus aureus*. In: Proceedings of the 7th SB Kurhaus workshop on antibiotics: "Epidemiological behaviour of resistant micro-organisms", December 10, 1993. Ed. van der Meer JWM, Michel MF, Verbrugh HA.

11 **Kluytmans J and Manders M.** Registratie en preventie van postoperatieve infecties in thoraxchirurgie. *Tijdschr Hyg Infectieprev* 1994;**13(1)**:5-8.

12 **Kluytmans JAJW, Mouton JW, Maat APWM, Manders MAAJ, Michel MF, and Wagenvoort JHT.** Surveillance of postoperative infections in thoracic surgery. *J Hosp Infect* 1994;**27**:139-47.

13 **Buiting AGM, Kabel PJ, Kluytmans J, Mouton JW en Voorn GP.** Automatisering en medische microbiologie: Resultaten van een landelijke inventarisatie. *Ned Tijdschr Med Microbiol* 1994;**2**:31-4.

14 **Kluytmans JAJW, Mouton JW, IJzerman EPF, Maat APWM, Vandenbroucke CMJE, Wagenvoort JHT, and Verbrugh HA.** Nasal carriage of *Staphylococcus aureus* as a major risk factor for the development of wound infections after cardiac surgery. *J Infect Dis* 1995;**171**:216-9.

15 **Veen A van 't, Mouton JW, Gommers D, Kluytmans JAJW, Dekkers P and Lachmann B.** Influence of pulmonary surfactant on in vitro bactericidal activity of amoxycillin, ceftazidime and tobramycin. *Antimicrob Agents Chemother* 1995;**39**:329-33.

16 **Kluytmans JAJW, van Leeuwen WB, Goessens WHF, Hollis RA, Messer SA, Herwaldt LA, Bruining HA, Heck M, van Leeuwen N, Rose J, van Belkum A, and Verbrugh HA.** Food initiated outbreak of methicillin resistant *Staphylococcus aureus*, analyzed by pheno- and genotyping. *J Clin Microbiol* 1995;**33**:1121-8.

17 **Bakker S, Kluytmans J, den Hollander J, and Lie S.** Subdural empyema caused by *Escherischia coli*. Hematogenous dissemination to a preexisting chronic subdural hematoma, a case report. *Clin Infect Dis* 1995;**21**:458-9.

18 **Rijsoort-Vos JA van, Stolz E, Verbrugh HA, and Kluytmans JAJW.** In vitro activity of a new quinolone (CP-99219) compared to ciprofloxacin, pefloxacin, azithromycin and penicillin against *Neisseria gonorrhoeae*. *J Antimicrobial Chemother* 1995;**36**:215-8.

19 **Goessens WHF, Kluytmans JAJW, den Toom NA, van Rijsoort-Vos JA, Niesters HGM, Stolz E, Verbrugh HA and Quint WGV.** Influence of the sample volume processed on the detection of *Chlamydia trachomatis* in urogenital samples by PCR. *J Clin Microbiol* 199;**33**:251-3.

20 **Van Belkum A, Kluytmans J, van leeuwen W, Bax R, Quint W, Peters E, Fluit A, Vandenbroucke-GraulsC, van den Brule A, Koeleman H, Melchers W, Meis J, Elaichouni A, Vaneechoutte M, Moonens F, Maes N, Struelens M, Tenover F and Verbrugh H.** Multicenter evaluation of arbitrarily primed PCR for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1995;**33**:1537-47.

21 **Voss A, Kluytmans J, and Vandenbroucke-Grauls C.** Hospital epidemiology and infection control in the Netherlands. *SHEA news* 1995;**2**:3-5.

22 **Van Belkum A, van Leeuwen W, Kluytmans J, en Verbrugh H.** Typeren van ziekenhuispathogenen. Moleculair biologische procedures met een gebruiksaanwijzing. *Tijdschrift voor hygiëne en infektiepreventie* 1995;**2**:53-6.

23 **van Belkum A, Kluytmans J, van Leeuwen W, Goessens W, ter Averst E, and Verbrugh H.** Investigation into the repeated recovery of coagulase negative staphylococci from blood taken at the end of cardio-pulmonary by-pass. *J Hosp Infect* 1995;**31**:285-93.

24 **van Belkum A, van leeuwen W, Kluytmans J, and Verbrugh H.** Moleculair nosocomial epidemiology: High speed typing of microbial pathogens by arbitrary primed PCR assays. *Infect Control Hosp Epidemiol* 1995;**16**:658-66.

25 **Koeleman JGM, Kluytmans JAJW, Vandenbroucke-Grauls CMJE, Verbrugh HA, en Voss A.** Hygiene en epidemiologie: Balans tussen medisch microbioloog en ziekenhuishygienist. *Nederlands Tijdschrift Med Microbiol* 1995;**3**:49-51.

26 **Koeleman JGM, Kluytmans JAJW, Vandenbroucke-Grauls CMJE, Verbrugh HA, en Voss A.** Preventie en bestrijding van ziekenhuisinfecties: multidisciplinaire aanpak gewenst. *Medisch Contact* 1995;**45**:1447-8.

27 **Renders NHM, Kluytmans JAJW, Stolz E, en Verbrugh HA.** Gentamycine-huidzalf: 200 kilo teveel. *Ned Tijdschr Geneesk* 1995;**139**:2314-6.

28 **Yzerman EPF, Boelens HAM, Tjhie JHT, Kluytmans JAJW, Mouton JW, Verbrugh HA.** ΔAPACHE II for predicting course and outcome of nosocomial *Staphylococcus aureus* bacteremia and its relation to host defense. *J Infect Dis* 1996;**173**:914-9.

29 **Van't Veen A, Gommers D, Mouton JW, Kluytmans JAJW, Krijt EJ, Lachmann B.** Exogenous pulmonary surfactant as a drug delivirimg agent: influence of antibiotics on surfactant activity. *Brit J Pharmacology* 1996;**118**:593-8.

30 **Luyendijk A, van Belkum A, Verbrugh H, Kluytmans J.** Comparison of five tests for identification of *Staphylococcus aureus* from clinical samples. *J Clin Microbiol* 1996, in press.

31 **Leverstein-van Hall MA, Dorigo-Zetsma JW, Mouton JW, Kluytmans JAJW, Voorn PG, Vos MC.** Landelijke enquete-'Typeringstechnieken in de ziekenhuisepidemiologie'. *Ned Tijdschr Med Microbiol* 1996;**4**:6-8.

32 **Kluytmans JAJW.** Surgical infections including burns. In: Wenzel RP, ed. Prevention and control of nosocomial infections. Williams and Wilkins, 3rd ed 1996. In Press.

33 **Bleker OP, Wigersma L, Kluytmans J.** *Chlamydia trachomatis* infectie. In: Bleker OP, Wigersma L, Coutinho RA, van Klingeren B, Laga M, Stolz E, eds. Compendium seksueel overdraagbare aandoeningen. Wetenschappelijk uitgeverij Bunge, 1996;65-73.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 19 juni 1962 te Oisterwijk. In 1980 werd het diploma Atheneum B gehaald aan het Sint Odulphus Lyceum, te Tilburg. In dat jaar werd de studie geneeskunde aangevangen aan de Erasmus Universiteit te Rotterdam. Het artsexamen werd op 20 november 1987 behaald. Van januari 1988 tot maart 1989 werd de militaire dienstplicht vervuld als medisch officier bij de Koninklijke Luchtmacht. In juli 1989 werd de opleiding medische microbiologie aangevangen (Opleider Prof. Dr. M. F. Michel) in het Academisch Ziekenhuis Rotterdam. Deze opleiding werd op 1 juli 1993 voltooid. Vanaf deze datum was hij staflid van de afdeling bacteriologie van het Academisch Ziekenhuis Rotterdam en als zodanig hoofd van de afdeling ziekenhuishygiëne. Deze positie werd vervuld tot 1 februari 1995, waarna hij toetrad tot de Maatschap Artsen-microbioloog Brabant. De werkzaamheden vinden plaats in de Ziekenhuizen van Breda, te weten het Ignatius Ziekenhuis, het ziekenhuis de Baronie en het Medisch Centrum de Klokkenberg.
De in dit proefschrift beschreven studies werden aangevangen gedurende de opleiding tot medisch microbioloog op de afdeling bacteriologie in het Academisch Ziekenhuis Rotterdam.